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ASSESSMENT AND IMPLICATIONS OF BACTERIAL REGROWTH IN WATER DISTRIBUTION SYSTEMS

by

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16. ABSTRACT

Two water distribution systems were studied over a 1-year period, Temporal fluctuations in a number of physical, chemical and biological parameters were examined. Total and pigmented bacterial counts, total coliforms, and fecal coliforms were determined at four locations within each distribution system on a bimonthly basis and on a monthly basis at source locations. Bacerial genera at the source and one site location in each system were identified at quarterly intervals. Three different nutritional media and three plating techniques were used to enumerate bacteria. M-Standard Plate Count medium followed by low nutrient R-2A medium yielded the highest bacterial counts across sampling locations. Lowest counts were obtained with the pour plate method. Physical parameters of redox potential, pH, dissolved oxygen, temperature and turbidity had little effect on total and pigmented bacterial counts in either system. Particle counts and particle size distribution showed a higher positive correlation with bacterial numbers in the untreated groundwater system (Garden Grove) than in the treated surface (Irvine Ranch) system. Total cations and anions, magnesium, calcium, and nitrate also showed a positive correlation with bacterial numbers. Residual chlorine showed a negative correlation with bacterial counts in the treated system. Studies indicated that bacterial isolates recovered from the treated system had a higher resistance to combined chlorine (disc assay technique) than did isolates from the untreated groundwater system. Scanning electron microscopy demonstrated bacteria associated with pipe surfaces, and with about 1 to 17 percent of suspended particles in the untreated groundwater system. Bacterial counts increased from source to site locations in both distribution systems, suggesting that regrowth occurred. The largest increases were noted in the unchlorinated Garden Grove System, but the magnitude of increases was site dependent.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This investigation reports bacterial regrowth in water distribution systems. The findings indicate that bacterial numbers increase in both chlorinated and unchlorinated distribution systems as water is transported from its source to the consumer. This deterioration is of importance in maintaining high quality and safe drinking water. Further, numerous bacterial genera which are defined as secondary opportunistic pathogens as well as those which cause taste and odor problems have been isolated. Turbidity and, more specifically, certain size ranges of particles increase as bacterial numbers increase. These findings indicate the need of a standard plate count to insure bacterial quality in water supply systems.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

Two water distribution systems were studied over a 1-year period. The Garden Grove Water Distribution system received groundwater with no treatment or chlorination. The Irvine Ranch Water District Distribution system received fully treated and chlorinated surface water.

Temporal fluctuations in a number of physical, chemical and biological parameters were examined. Total and pigmented bacterial counts, total coliforms, and fecal coliforms were determined at four locations within each distribution system on a bimonthly basis and on a monthly basis at source locations. Bacterial genera at the source and one site location in each system were identified at quarterly intervals. Three different nutritional media and three plating techniques were used to enumerate bacteria. M-Standard Plate Count medium followed by low nutrient R-2A medium yielded the highest bacterial counts across sampling locations. Lowest counts were obtained with the pour plate method.

Physical parameters of redox potential, pH, dissolved oxygen, temperature and turbidity had little effect on total and pigmented bacterial counts. Particle counts and particle size distribution showed a higher positive correlation with bacterial numbers in the Garden Grove System than in the Irvine system. Specific chemical constituents in the water also correlated with bacterial numbers. Total cations and anions, magnesium, calcium, and nitrate seemed to be most important in this respect.

In addition, residual chlorine correlated negatively with bacterial counts in the Irvine system. Chlorine resistance studies indicated that bacterial isolates recovered from the Irvine System had a higher resistance to combined chlorine using a disc assay technique than did Garden Grove isolates.

Scanning electron microscopy demonstrated bacteria associated with pipe surfaces, suspended, and particulate matter in the Garden Grove System. Approximately 1 to 17 percent of such particles had associated bacteria.

Bacterial counts increased from source to site locations in both distribution systems, suggesting that regrowth occurred in both distribution systems. The largest such increases were noted in the unchlorinated Garden Grove System, but the magnitude of increases was site dependent.

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LIST OF ABBREVIATIONS

AC -- Asbestos cement pipe
BGB -- Brilliant green bile broth
BLS -- Bacteriocin-like substances
C -- Concrete pipe
CONF-col -- Confirmed MPN coliform
EC -- Fecal coliform broth
EDX -- Energy dispersive x-ray analysis
EMB -- Eosin methylene blue agar
Fecal-EC -- Fecal MPN coliform
GI -- Galvanized iron pipe
GG -- Garden Grove Water District
IRWD -- Irvine Ranch Water District
LTB -- Lauryl tryptose broth
M-endo -- Membrane filtration presumptive coliform
M-FC -- Membrane filtration fecal coliform
MPN -- Most probable number
m-SPC -- Membrane filtration standard plate count
NS -- Not statistically significant
NTU -- Nephlometric turbidimeter units
O-F -- Basal medium - oxidative fermentative sugars
PAH -- Purgeable aromatic hydrocarbons
PC -- Plate count agar
PHO -- Purgeable halogenated hydrocarbons
PRES-col -- Presumptive coliform - MPN coliform
R-2A -- Low nutrient R2-A medium
S -- Steel pipe
SEM -- Scanning electron microscope
SRCC -- Spearman Rank Correlation Coefficient
TC-MFU -- Membrane filtration total coliform - unconfirmed
TC-MFC -- Membrane filtration total coliform - confirmed
TDS -- Total dissolved solids
TOC -- Total organic carbon
TPC20PP -- Total plate count 20°C - pour plate
TPC35PP -- Total plate count 35°C - pour plate
TPC20SP -- Total plate count 20°C - spread plate
TPC35SP -- Total plate count 35°C - spread plate
TPCP20PP -- Total plate count pigmented 20°C - pour plate
TPCP35PP -- Total plate count pigmented 35°C - pour plate
TPCP20SP -- Total plate count pigmented 20°C - spread plate
TPCP35SP -- Total plate count pigmented 35°C - spread plate

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SECTION 1

INTRODUCTION

The importance of waterborne disease outbreaks has been a concern of public health officials since the inception of public health microbiology. Today, few citizens realize that the incidence of waterborne disease has risen sharply since the 1950's. Etiological agents have been identified in only 43% of 192 disease outbreaks during the period of 1971-77 (29). Semipublic water systems were associated with 58% of the total outbreaks, and municipal systems accounted for another 30%.

These two types of systems accounted for 88% of the total cases of waterborne disease from 1971-77 (29). The exact reasons for these increases are still not well understood and may involve the completeness of reporting of waterborne diseases before 1971.

Out of the English experience in the 1800's with the devastating waterborne epidemics of cholera and typhoid the principles of water works practice were developed. The primary objective is to obtain the purest water source. The second is to purify the water in the best possible manner, and the third is to protect the water during its distribution. With respect to bacteriological water quality, the water source should have low bacterial concentrations, and treatment must be adequate to remove bacteria. For surface water sources, properly operated treatment processes such as sedimentation, filtration, and chlorination lower the bacterial counts to acceptable levels. Normally for ground waters, disinfection by chlorination is the only treatment required to attain an acceptable bacteriological quality.

The use of a chlorine residual for maintaining excellent bacteriological quality during water distribution is the traditional water treatment practice in the United States, and it is still recommended by the American Waterworks Association.

Maintaining an effective chlorine residual in the distribution system is the primary means of preventing bacterial regrowth. However, reduced chemical compounds such as H_2S, Fe^{+2}, NH_3, and organics in the distribution system exert a chlorine demand (36). The presence of these compounds in a water system reduces the chlorine residual, thereby allowing bacteria to grow. These chemical compounds may originate from the source water or enter the system as a result of a physical break, corrosion, cross connection, or routine maintenance. The physical break can be from poor structural features in the joints, sudden traumatic impact, or internal and external pipe corrosion.

Corrosion can play a significant role in bacterial regrowth for several reasons. Foremost, Fe^{+2} ion is a product of iron pipe corrosion and capable of exerting a chlorine demand. Normally Fe^{+2} is readily oxidized to $Fe(OH)_3$, by oxygen present in the water, so that the level of total iron does not significantly increase. This reaction, however, takes place on the side of the pipe. In addition, scale and pitting as a result of corrosion provide microhabitats and additonal surfaces for bacterial attachment. The bacterial colonization of such habitats has been noted by Allen and Geldreich (4). Corroded sections of water mains have also been shown to be microbiologically active (1). Though the occurrence of microorganisms in water distribution systems has been known for centuries, these recent works suggest a diverse and intricate series of microhabitats within distribution systems that are capable of supporting bacteriological growth. The understanding of these habitats and their formation lies to a great extent in the chemistry of water, with corrosion parameters exerting a major influence.

One of the primary objectives of this research was to determine the nature and extent of association of bacteria and other kinds of microorganisms with the inner surfaces of water mains and with suspended particulate matter (e.g., silt and detritus particles). Fouling of pipe surfaces as a result of the growth of microbial films or inorganic mineral deposition can be a major factor in the premature deterioration of distribution lines and represents a significant and continuing economic problem, especially in older systems (31, 57).

One of the factors affecting colonization of pipe surfaces is the ability of a bacterium to adhere to a solid surface. Attachment increases its access to trace quantities of minerals and various organic nutrients, especially in oligotrophic environments where these substances tend to be concentrated at solid-liquid interfaces (80). In addition, cells that are intimately associated with the interior walls of water mains may be less susceptible to chemical disinfection processes because of boundary layer effects and the secretion of extracellular coatings (11, 133). As a result, the extent to which microbes become associated with the inner surfaces of pipes or with suspended particulate matter within the water column could significantly enhance their survival and regrowth potential in distribution lines and reservoirs.

To obtain information concerning the nature and extent of association of microorganisms with solid interfaces in distribution systems, the surfaces of pipe fragments and suspended particulate matter were examined in the scanning electron microscope. This technique has been frequently used to determine the *in situ* microzonal distribution of attached microorganisms on a wide variety of environmental surfaces (59, 94, 100). Because the chemical composition of the pipe surface may influence microbial activity, elemental constitution of the pipe surface was also investigated by x-ray energy dispersive microanalysis.

The fundamental reason for the corrosion of metals used in water supply conduits is that they are thermodynamically unstable in the normal aqueous environment. Given enough time they will dissolve. Under the Eh and pH normally found in potable water systems, the stable species is Fe_2O_3 and not Fe^0. The long-term tendency will be for all the iron to convert to this stable form.

The rate of this conversion process is in part enhanced by a number of water quality parameters. There is increasing evidence indicating that the chloride ion is the major aggressive (accelerates corrosion) ion. In certain situations the sulfate ion is also aggressive. The bicarbonate and nitrate ions are considered unaggressive (passivates corrosion).

Where domestic water is concerned, the principle mechanism of corrosion inhibition is through the manipulation of water chemistry to encourage the formation of scale on the cathodic region. Most oxidizing inhibitors, such as nitrate or chromate would not be acceptable in domestic water supplies. Under the conditions in which most utilities operate, materials intended for anodic scale formation would increase the risk of pitting to unacceptable levels.

Probably the most widely recognized scale formation parameter is the calcium carbonate saturation index, developed by W.F. Langelier (69). Langelier's proposal was a systematic attempt to predict the degree of calcium carbonate saturation of a water supply given the water analysis. Its relationship to corrosion was based on early observations by Tillmans (124) that, when slightly super-saturated, $CaCO_3$ would form an eggshell lining on the inside of iron pipes, thus reducing red water problems, hence corrosion.

Although some have attempted (71, 119) no one has ever successfully developed a direct correlation between the Langelier index and the corrosion rate. McCauley (77) even developed a process for coating pipe with a $CaCO_3$ lining. However, a Langelier index adjusted for slight supersaturation, does not insure that a uniform $CaCO_3$ scale will necessarily form. There are three important cases where such a scale does not form, namely, conditions of low velocity, high pH, and high sulfate.

The importance of velocity to the formation of proper scale is easily grasped. At low velocities the scale formed is too porous and nonuniform. Such a scale will not reduce the rate at which oxygen reaches the cathodic surface. McCauley showed that velocities of 1 fps or more are necessary to develop a scale with satisfactory integrity. This means that at low flow and dead end portions of a system corrosion problems are more pronounced. Also, if a scale develops it is more porous. The Langelier index is also less useful when high pH's are necessary to get a positive value. First, at high pH values, both scaling and corrosion phenomena are less uniform in character (119). As a result, tuberculation is increased at high pH values (70, 71). Second, insufficient calcium and alkalinity are present in these waters to deposit enough $CaCO_3$, for adequate corrosion protection. The low calcium and alkalinity are also the reason that the pH must be increased to such a high level to make the Langelier index positive. Finally at pH's significantly above 9, orthosilicic acid becomes a factor changing the chemical nature of the deposit.

When a water possesses a high sulfate content, the calculations ordinarily used for the Langelier index can also be adversely influenced due to the formation of soluble $CaSO_4^0$. A good example is natural Colorado River water as delivered by the Metropolitan Water District in 1970 (Ca = 87 mg/l, SO_4 = 326 mg/l). Solving the appropriate equilibria equations will demonstrate that 35% of the calcium in this water is tied up as $CaSO_4^0$. Whereas Langelier's equation gives an Langelier index of +0.23, the actual index, corrected for $CaSO_4^0$, is only 0.04.

In spite of the above, the Langelier index is still the best available measure for evaluating a water's potential for forming $CaCO_3$ scale. It is thermodynamically sound and by virtue of its direct relationship with the free energy driving force it should bear some relation to the kinetics of calcium carbonate precipitation as well as resolving the question of supersaturation (89).

The amount of pH adjustment required depends on the net amount of alkalinity-reducing chemicals used during upstream treatment, however, the requirements can be accurately predicted using a simple computer program (123). Examples of possible alkalinity consuming chemicals are copper sulfate, chlorine, alum, and hydro-flourosilicic acid.

The mediation of corrosion by the Langelier index can predict the availability of microhabitats and irregular surfaces conducive to bacterial attachment. In addition, waters slightly over-saturated with $CaCO_3$ can promote formation of inorganic precipitates instead of the scale on the pipe. This floc can entrap and protect bacteria which could then settle out in dead ends and reservoirs.

Certain microorganisms such as the iron oxidizing bacterium Gallionella are also known to promote corrosion processes in iron pipes. Very little is known about the biology of Gallionella, or other kinds of iron bacteria, despite their economically important roles in initiating and perpetuating biogeochemical corrosion processes in water distribution systems. Hanert (50) observed measurable fixation of $^{14}CO_2$ by G. ferruginea grown axenically in a $^{14}CO_3$-FeS medium. These data as well as other less direct physiological evidence (48, 67), suggest that Gallionella, as well as certain other iron bacteria (27, 122) derive their essential energy requirements through a strictly chemolithotrophic process, namely the enzyme-mediated oxidation of ferrous (and sometimes also manganous) ions with the concommitant fixation of carbon dioxide. This typically results in the precipitation about the cell and stalk of ferric salts, usually the hydroxide form, which renders the cells brown or reddish-brown. Under favorable conditions, Gallionella organisms can rapidly multiply in water distribution systems resulting in discoloration of the water and eventual impairment of the flow of water through the pipes due to the massive accumulation of insoluble iron salts. Such heavy growths of iron bacteria can alter the physio-chemical properties of the pipe surfaces and themselves serve as nutritional substrates for various chemoorganotrophic microorganisms. The metabolic activities of the latter under anaerobic conditions can produce organic acids and hydrogen sulfide which in turn can lead to taste, odor, and corrosion problems. Reports of fouling and corrosion of wells and water distribution mains by Gallionella have increased since the 1940's and certain investigators have suggested this is due to an increased occurrence of iron bacteria in these systems.

Another area of concern is the presence of turbidity. Turbidity is an indirect measurement of the particulate matter in water. The measurement of turbidity provides no information as to the type of particles or the distribution of particles in the water. The rationale for including turbidity as a primary drinking water standard was based on its interference in the disinfection process (120). Examination of the experimental data presented shows that organic, cellular turbidity interferes with the disinfection process but that inorganic turbidity does not. Others have reported that inorganic turbidity can entrap organisms, protecting them from the disinfection process (25, 109).

Bacteria associated with suspended particulate matter have been described in many natural aquatic environments including seawater, freshwater lakes and streams (42, 52, 80, 94). Research on bacteria attached to particles in these various aquatic environments has primarily focused on their ability to metabolize and cycle nutrients in these systems (5, 3, 13, 96).

Recently, our laboratory has observed numerous bacterially-colonized particles from unchlorinated municipal groundwater sources. Normal bacteriological techniques for the evaluation of potable water systems for the purpose of sanitary quality control fail to take into account the importance of such bacteria-laden particles. Utilizing standard plate count methods a particle bearing anywhere from several hundred to several thousand attached microorganisms would appear as a single bacterial colony and thus be assumed to have arisen from one bacterial cell.

The potential for bacteria-laden particles to cause disease in the human population is problematic. However, the ingestion of a few such bacteria laden particles may establish a dose sufficient to elicit a pathological response on the part of the host if the attached microorganisms possessed pathogenic qualities. It is also possible that bacteria aggregated on the surfaces of suspended particulate matter might be significantly more resistant to conventional modes of disinfection, such as chlorination, especially if extracellular mucopolysaccharide substances are secreted by the cells.

Once past the disinfection process of the treatment system, the nature of turbidity has a role in bacterial regrowth. Some inorganic particulate matter may exert a chlorine demand thereby decreasing the bacterial barrier. In addition, the presence of entrapped organisms surviving the disinfection process and settling out in the distribution system would serve as an inoculum for regrowth problems.

Another manner in which water chemistry can influence regrowth is in systems where waters of very different qualities are blended; for example, Colorado River water, EC = 1100 micromhos - cm^2, and local well water EC = 300 micromhos - cm^2. In such blends ionic strength becomes an important factor since the surface charge of an organism can be altered. Aggregation of bacteria and viruses occur as ionic strength increases (130). The clumping of microorganisms has been shown to alter the kinetics of disinfection. The net result is that under the same conditions the aggregated forms are more resistant (63, 113).

Chemical and physical factors within a distribution system have been shown to affect the numbers of bacteria occurring in the system. Some of these factors include temperature, residual chlorine levels, turbidity, pulsation in water pressure, and the type of pipe in service (43). The role of chemical factors in bacterial regrowth in water distribution systems has received little attention. Bacterial regrowth in the presence of chlorine residuals of 0.5 ppm indicate that the bacteria present are being protected or are more resistant to chlorine. Some of the aforementioned chemical phenomena could enhance the survival of bacteria in the system.

Despite recent advances in knowledge concerning physiological mechanisms of chlorine disinfection, all of the physico-chemical and biological parameters which influence the bactericidal properties of chlorine in the environment are not yet fully understood. Large numbers of viable microorganisms can often be recovered from potable water distribution systems where a free chlorine residual of 0.5 to 1.0 mg/L is maintained (14, 83). Thus specific mechanisms may exist for the survival of bacteria in waters containing relatively high concentrations of chlorine. Further, evidence has been reported suggesting that repeated exposure of certain microorganisms to chlorine may lead to physiological or genetic selection for increased resistance to this halogen. Some proposed mechanisms for bacterial (or viral) resistance to chlorine include: (i) aggregation or clumping of cells *in situ*; (ii) attachment of microbes to pipe surfaces or to suspended particulate matter such as detritus or clay particles; (iii) extrusion of protective extracellular capsular or slime layers (111); and (iv) formation of resistant spores. Conceivably, some of these mechanisms could favorably influence the survival of indicator or pathogenic microorganisms in drinking water distribution systems.

If chlorine-resistant microbial forms exist, they should be preferentially selected for in chlorinated water distribution systems. Conversely, bacterial populations residing in unchlorinated systems should, on the whole, be skewed toward increased sensitivity to chlorine disinfection, since more resistant cell types would not exhibit any special selective advantage under such circumstances.

Evaluation of Total Plate Count Techniques

Since its inception, public health microbiology has been concerned with insuring a bacteriologically-safe water supply. With a multitude of media and methodologies, microbiologists have worked to prevent the occurrence of disease epidemics. Due to the difficulty involved in detecting pathogens directly, their effort has primarily focused on the detection of indicator bacteria within water supplies. These bacteria suggest the potential presence of intestinal-associated pathogens in the water. Unfortunately, the inferred relationship between indicator bacteria and pathogens has not always proven valid. Riverside, California, experienced an outbreak of Salmonellosis in 1965. *Salmonella typhimurium* was isolated from six different samples of municipal water. Interestingly, Salmonellae were ten times as numerous as the common indicator organism, *Escherichia coli* (15). In another instance, seven different strains of *Salmonella* were isolated from treated and untreated drinking water in eleven of 2,128 water samples in France (115). Counts of *Escherichia coli*, coliforms and streptococci met bacterial standards in nine of these eleven instances.

Occasional failure of the coliform group to satisfactorily identify potential disease threats has prompted investigation into new and more effective indicator groups. However, so far a suitable alternative bacterial indicator group has not been found and the large volume of historical data on coliforms in drinking water continues to make them the primary measure of a safe drinking water supply.

In light of low coliform incidence in water distribution systems, the United States Environmental Protection Agency has endorsed the use of the Standard Plate Count (SPC) as a supplemental evaluative tool of bacteriological water quality (121). The Standard Methods pour plate procedure (118) has come under increasing criticism from opponents who cite the selective effects of heat shock and low oxygen tension (64, 127) inherent in the method. Foremost, Clark (23) has shown the spread plate method to be superior in accuracy to the pour plate procedure. It is clear that plating methodology is one of the important barriers to developing a bacterial recovery system which accurately reflects microbial conditions in water distribution systems.

Innovations in plate count methodology. The recent development of two new media for the recovery of total heterotrophic bacteria in water distribution systems has further increased the potential for enhanced bacterial recovery in water supplies. Taylor and Geldreich (121) have developed a standard plate count medium (m-SPC) which is superior to plate count medium (PC, Difco). Parallel studies using both laboratory and commercially prepared m-SPC medium have produced bacterial counts as high or higher than those obtained with the Standard Methods pour plate technique (118). Further, the m-SPC membrane filtration method confers the advantage of processing large sample volumes. Although this procedure is not currently a standard method, it offers a viable and improved alternative to present methodologies.

The composition of media has long been used to select for various distinct bacteriological populations. It is becoming increasingly evident that non-selective media may in fact be quite selective. Reasoner and Geldreich (101) have shown that bacteria in water distribution systems can be recovered with greater efficiency on a medium containing small amounts of the essential growth metabolites rather than on a nutrient rich medium. This low nutrient formulation has, in certain instances, recovered higher counts of bacteria and percentages of pigmented bacterial forms in comparison to Tryptone Glucose Yeast agar (101).

Due to requisite long incubation times, pigmented bacteria are often overlooked in routine laboratory plate counts. However, they are now being examined more closely as potential opportunistic pathogens in water.

As more investigations on the types of organisms present in water distribution systems are reported, the number of genera recovered increases. Geldreich et al. (46) have divided water distribution bacterial isolates into four categories: coliforms, coliform antagonists, opportunistic pathogens and other organisms. Lee (74) have reported the isolation of *Arthrobacter*, *Flavobacterium*, *Enterobacter*, *Pseudomonas*, *Actinomyces*, *Azotobecter*, *Bijerinckia*, yeast, acid-fact bacilli and sulfate reducing and oxidizing organisms. The level of residual chlorine appears to be critical with highest counts occurring when residual chlorine is lost (129).

Bacterial regrowth may seriously hinder the detection of coliforms in a water distribution system. Therefore, a thorough assessment of the bacteria present in water distribution systems will aid in insuring better quality.

Hutchison et al. (56) demonstrated that values of the most probable number for E. coli were reduced from 28% to 97% by Pseudomonas, Actinomyces, Sarcina, Micrococcus, and Flavobacterium. This work along with that by Reitter and Seligmann (104) indicated that coliforms can be directly inhibited by other bacteria during the enrichment phase of the coliform test or through contact with the bacteria for a period of time prior to inoculation into the presumptive portion of the test. High densities of Klebsiella have also been shown to suppress E. coli (99). Data from the literature indicate that high densities of noncoliform bacteria (500/ml) can cause coliform suppression (76). Waksman (131) reviewed the literature examining the different mechanisms that microorganisms utilize to inhibit other bacterial populations. Two or more organisms living in close proximity will exert antagonistic, indifferent, of favorable effects on the other population. Pigment formation by P. aeruginosa may be weakened in the presence of other organisms, and E. coli may lose the ability to ferment lactose. This latter point is of primary significance in the coliform test since a positive reading is dependent on the fermentation of lactose in the most probable number method. Though other coliform organisms have not been examined in this respect one might hypothesize that lactose fermentation could be similarly impaired through competition with other bacteria. From the data presented in the literature both bacterial density and the type of bacteria present appear to be of primary importance in coliform suppression, and that suppression could occur through two modes. The first would be through the inhibition or damage to the coliform prior to its inoculation into the presumptive medium of the coliform test resulting in the coliforms' inability to ferment lactose and the second through competition for the substrate within the presumptive medium itself. It is highly plausible that both occur in environmental situations. However, to date, there is a paucity of data distinguishing these two phenomena.

Pseudomonas, Bacillus, Streptomyces, Micrococcus, Flavobacterium, and Proteus have all been isolated from standard plate counts (24, 55, 75, 127). Many of these bacteria can inhibit various coliform bacteria (primarily E. coli). This would indicate that the potential for the suppression of coliforms is often present in water distribution systems. And that it may manifest itself when densities of the aforementioned organisms reach 500 bacteria/ml or when specific genera of bacteria are present in sufficient numbers to elicit inhibitory effects.

Bacteriocins were discovered in 1925 (40) when it was observed that the supernatant from a broth culture of one strain of the bacterium Escherichia coli would not support the growth of another strain, even when diluted a thousand-fold. Further experimentation by Gratia (40) and others, notably Frederiqc (40), led to the discovery of many different colicins (bacteriocins specifically inhibitory for E. coli) and the development of a typing scheme.

Classically, bacteriocin-like substances (BLS) have been studied from a molecular-genetic perspective. Several excellent reviews of the bacteriocin literature give detailed accounts of the biochemical and molecular processes involved in colicin production and activity (17, 58, 91, 102, 103). Two distinct types of bacteriocins have been identified. Both have been shown to be proteins (103). One is a small molecular weight protein (usually $< 60,000$ daltons) which cannot be readilly sedimented by ultracentrifugation and has not been visualized using electron microscopy. The other is a large molecule ($> 10^6$ daltons) easily sedimented, and in electron micrographs closely resembles a bacteriophage tail assembly in morphology (103). Customarily, bacteriocins have been named for the organisms from which they were isolated; thus, *E. coli* produces colicins and *Serratia marscesens* produces marscesins.

Early research indicated bacteriocins were fatal to the cell once adsorption to the cell surface occurred; however, this effect was reversible if the bacteriocin was cleaved with trypsin within two hours of bacteriocin adsorption at the sensitive cells' receptor (91). This suggested that some bacteriocins may be bacteriostatic in nature (17).

The influence of non-coliform microorganisms on the survival and recovery of coliform bacteria has been well documented in the literature. In freshwater environments, antagonism of *E. coli* by non-coliform bacteria has been shown both directly and indirectly. Hutchison et al. (56), isolated bacteria capable of inhibiting *E. coli* in 8, 11 and 37% of water samples from spring, groundwater and surface sources, respectively. By adjusting the densities of these antagonistic bacteria, they were able to show a reduction in the recovery of *E. coli* after incubation for 12 hours at 10°C. Enumerations of *E. coli* in these suspensions by the Most Probable Number (MPN) technique (118) were reduced by 28 to 97%. However, these experiments did not differentiate between the phenomena of coliform injury (14) and death.

The experimental results of Hutchison et al. (56) were corroborated by data from the National Water Supply Study (76) as analyzed by Geldreich et al. (43). Coliform detection in the water distribution systems studied was stable as non-coliform bacterial numbers increased up to 500 cells/ml. At densities of 1000 non-coliform cells/ml, incidences of coliform detection decreased in frequency. Other researchers have reported similar findings (104).

Various studies have implicated specific genera as being "antagonistic" toward coliform bacteria. These include *Psuedomonas*, *Sarcina*, *Micrococcus*, *Flavobacterium*, *Proteus*, *Bacillus* and *Actinomycetes* (37, 65, 132).

Three basic phenomena may explain the interaction of non-coliform bacteria with coliform bacteria and the subsequent suppression of the coliforms in standard MPN tests: nutritional competition, injury, or chemical antibiosis. The phenomenon of bacterial injury(14) might exacerbate the effects of both nutritional competition and antibiosis sensitivity.

Of primary interest in this research is chemical antibiosis, specifically, the interaction of coliforms with bacteriocin-like agents or related inhibitory substances produced by non-coliform bacteria.

The mechanism of coliform suppression has not been fully determined. However, bacteriocins or bacteriocin-like substances (BLS) might be responsible for the occurrence of false-negative results (growth without gas production in the presumptive medium) in the MPN method (118). The failure of coliform bacteria to produce gas from lactose fermentation in the presumptive portion of the test, and the recovery of this ability in the confirmed test may be a function of bacteriocin adsorption and release. This "growth but no gas" phenomenon has been reported for seawater (92), freshwater, and drinking water samples (Olson, unpublished, 2). Suppression of coliform bacteria can result in a serious underestimation of the bacteriological quality of the water supply.

Further, Pseudomonas and Flavobacterium are of public health concern due to their role as secondary pathogenic invaders (30, 32, 88). Ptak et al. (99) found that 67% of the colonies selected from plates of treated water samples were Klebsiella. This extremely important finding indicates the existence of a known pathogen strain in the water supply. Therefore, it might be possible to have acceptable numbers of potential pathogens in the water supply.

The above literature would indicate that bacterial regrowth is a common problem in certain distribution systems. The data also indicate the need to elucidate further the impact of bacterial regrowth on the microbiological quality of potable water. Therefore, an investigation into factors which affect bacterial regrowth and coliform suppression in water distribution systems is a high priority in order to elucidate their impact on water quality. The second year of this study continued to identify bacteria occurring on standard plate counts in two distribution systems, and identify chemical and physical factors which are of importance in promoting bacterial regrowth. By delineating which factors are of primary importance in this phenomenon and examining the specific bacteria involved in terms of growth limiting factors and their influences on coliform suppression in the laboratory and field, it should be possible to develop methods which might alleviate the aforementioned problems. Through the application of information gained by bacterial identification and physical and chemical factors affecting the growth of these bacteria in distribution systems, it should be possible to apply this knowledge in field trials to reduce bacterial regrowth in water distribution systems.

SECTION 2

CONCLUSIONS

Several generalizations can be drawn from this study.

1. Bacterial counts and total particles increased from source to site in both distribution systems.
2. Bacterial counts were higher in the unchlorinated Garden Grove system.
3. M-Standard Plate Count and low nutrient R-2A media with 96 hour incubation periods yielded the highest total plate counts. The pour plate technique yielded the lowest total counts.
4. Certain chemical factors such as total cations and anions, NO_3, magnesium, and calcium covaried significantly with total plate counts on various media.
5. Physical factors, such as pH, temperature, redox potential, etc., with the exception of particle count and particle size distribution, had a limited effect on total plate counts.
6. Different media appeared to recover different bacterial populations as shown by pigmented counts obtained on the various media tested.
7. Scanning electron microscopy indicated that bacterial microcolonies as well as single bacterial cells were associated with galvanized pipe sections and free floating particles from the Garden Grove Distribution System.
8. Residual chlorine levels were negatively correlated with bacterial counts. However, bacteria were recovered continuously from the chlorinated Irvine System.
9. Bacteria isolated from the Irvine System were more resistant to combined chlorine than those isolated from Garden Grove.
10. Coliform suppression was demonstrated in 4.8% of MPN samples in the Garden Grove System.
11. The predominant genera at both source and site locations fluctuated throughout the course of the study.
12. Chemical parameters measured do not provide explanations of bacterial numbers nor genera.

SECTION 3

RECOMMENDATIONS

1. Development of new methods to detect injured coliforms in distribution waters.

2. As bulk water quality parameters have little predictive value in the behavior of microorganisms, new chemical parameters such as amino acids should be investigated in water supply systems to determine their effect on microbial regrowth.

3. A new method to assess total bacteria in water supply systems should be developed.

4. Efforts should be made to distinguish the significance of high counts versus the types of genera composing the population in water distribution systems.

5. The greater survival role of pigmented bacteria in chlorinated or stressed environments should be studied to determine what is regulating this phenomenon.

6. Chemical parameters should be investigated to determine if they can be used to predict the occurrence of certain bacterial genera.

7. The physiological condition and activity of bacterial and fungal populations *in situ* within water distribution systems should be determined.

8. Coliform suppression within distribution waters should be further studied to determine its mechanism.

9. More information is needed on the metabolic diversity and ecological status of bacteria within water distribution systems.

10. The elemental nature and source of bacterially colonized particles should be investigated.

11. Characterization of the metabolic activity of particulate attached bacteria and free bacteria within the supply system should be determined.

12. Factors protecting bacteria in water supply systems from disinfection should be studied in detail including particulate and turbidity interactions.

SECTION 4

EXPERIMENTAL PROCEDURES

Selection of Water Distribution Systems

Two water distribution systems were selected for this study: the Irvine Ranch Water District (IRWD) and the City of Garden Grove Water Distribution System. The IRWD system receives surface water from a combination of sources. Water enters the Diemer water plant from the Colorado River and from Northern California. These two sources are then blended accordingly to achieve a Total Dissolved Solids (TDS) measurement of 500 mg/l. Colorado River water has proportionately higher TDS level and therefore, the blend is generally 40:60 in favor of the Northern California water. This blended water receives full treatment at the Diemer Plant and is chlorinated after treatment. The water is then transported to the Irvine area and enters the distribution system at four turnouts.

The IRWD system services 75,000 people through 231 miles of water mains (2-24" in diameter). The system is relatively new, the oldest pipe being installed in 1962. Asbestos-cement represents 95% of the pipe material and steel cylinder lined with plastic, 5%. Some polyvinyl chloride (pvc) pipe is also present in this system. An average pipe pressure of 85 psi is maintained throughout the system.

The second water distribution system selected was in the City of Garden Grove and is supplied primarily with ground water obtained from a series of thirty wells in the Garden Grove area. In a normal year it receives 10% of its total supply from the Colorado River or from the Northern California Project Water (Metropolitan Water District of Southern California). This water arrives through six import connections and is stored along with the well water in seven reservoirs, four of which are below ground level. The collective storage capacity of these reservoirs is 45 million gallons. Surface waters are used only when peak demand overtaxes the delivery capabilities of the wells; this is most common during the summer. Normally, there is no treatment or chlorination of the water prior to its distribution into the supply system. However, when surface waters are mixed with the well water, chlorine residuals of approximately 0.1 ppm can be detected at several locations within the distribution system. This occurs primarily during the summer.

Pipe materials in this system are asbestos-cement, with galvanized iron present in a few of the older areas of the city. Galvanized iron represents less than 5.0% of the total pipe used in this system. The system serves approximately 150,000 persons through 377 miles of water main. Though the city has had no previous history of coliform problems, it was indicated to us that it might be a likely area for high bacterial counts.

System Evaluation

The purpose of the sanitary engineering evaluation was to review the operational and physical parameters of each system and determine their affect on bacteriological regrowth in the distribution system.

A distinct, well defined area of each system was selected for study. The selection was based on locating a portion of the distribution system that is served by a single source of supply, that is, one well or one MWD interconnection. For purposes of continuity, each system will be discussed separately.

The study area consists of the eastern portions of the Cities of Garden Grove and Irvine (Figures 1 and 2).

Site Selection

Initially, ten sites in Irvine were assessed for bacterial numbers once during July and four times during the month of August (Tables 1 and 2). Two media were used, total plate count (TPC) and trypticase soy agar (TSA) to estimate total counts. Bacteria were plated on both media by the spread plate method and incubated at 35°C for 48 hours. The bacterial counts shown in this initial phase are presented in Table 2. At the end of the month of August four sites were selected on the basis of high bacterial counts for in-depth study in Irvine. The sites included Paseo Picasso, Via Messina, L. Parkway Lane (L. denotes location of hydrant) and Arbor Lane. In January, 1979, sampling at the Irvine source (site feeder No. 4) was initiated. Beginning in July, 1980. sampling was only performed at Irvine Source and Paseo Picasso.

In Garden Grove thirteen sampling sites were selected for bacteriological screening during the initial phase of the study. The location of the sampling sites and the type of pipe material at each site are shown in Tables 3 and 4. Six locations in Garden Grove were selected for further study. Bacteriological total plate count data on these sites are shown in Table 4. Based on these numbers four sampling locations were selected for further study: Rockinghorse, Harris Street, Sandra No. 2 and Marshall Lane. During the second year of the study only Rockinghorse was sampled. Three well sites were examined during the study: well 6064, well 6321, and well 23/9 beginning January 1979, August 1979 and October 1979, respectively.

Sampling Regime

All samples were collected from fire hydrants. A flow meter was attached to the hydrant to allow the release of 200 gallons of water prior to sample collection. The hydrant was opened to release water at a rate of 200 gpm and run for 60 seconds. All on-site measurements other than bacteriological samples were taken using a special sampling device (Figure 3) at bimonthly intervals from May 1978 through June 1979 at Paseo Picasso, Via Messina, Left Parkview Lane, Arbor Lane, Rockinghorse, Harris Street, Marshall Lane, and Sandra No.2. Irvine source site no. 4 and Garden Grove wells 6321 and 6064

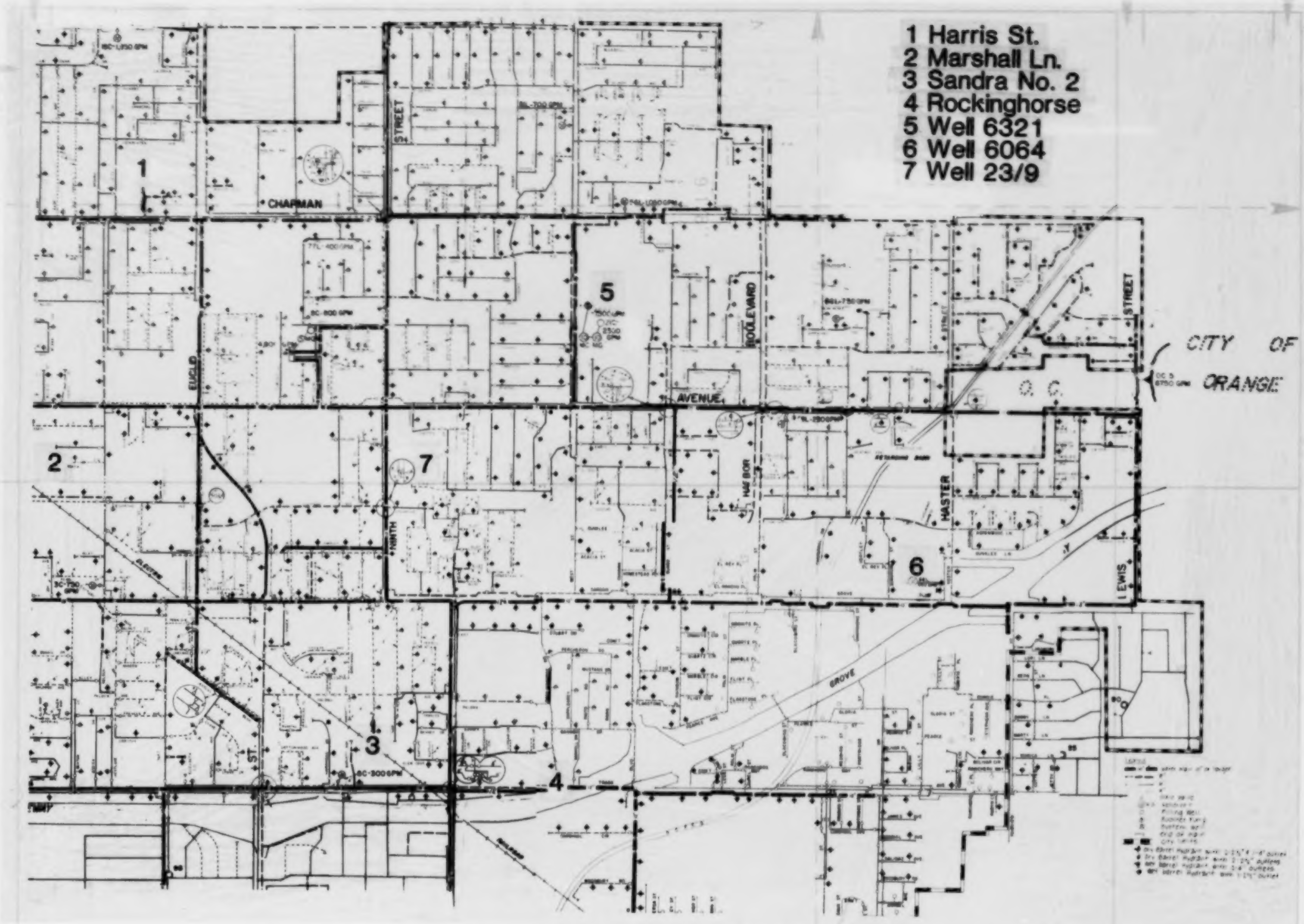
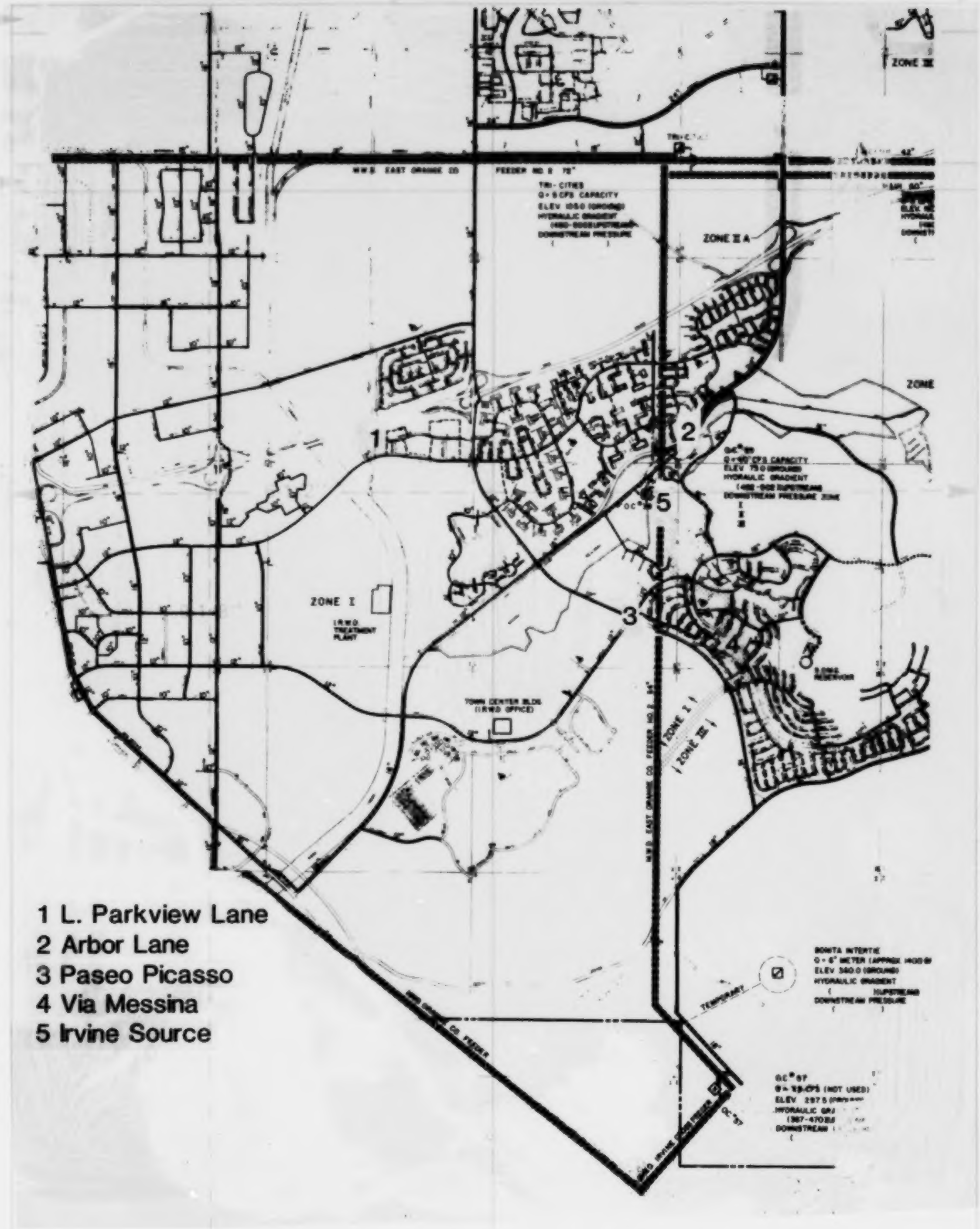

1 Harris St.
2 Marshall Ln.
3 Sandra No. 2
4 Rockinghorse
5 Well 6321
6 Well 6064
7 Well 23/9
CITY OF ORANGE
O. C.
CHARMAN
STREET
EUCLID
AVENUE
BOULEVARD
HARBOR
NINTH
HASTER
LEWIS
GROVE
ST
1
2
3
4
5
6
7

Figure 1. Sample Locations in the Garden Grove Distribution System.


M.W.D. EAST ORANGE CO. FEEDER NO 2 72"
TRI-CITIES
ZONE II A
ZONE III
ZONE
ZONE I
IRWD TREATMENT PLANT
TOWN CENTER BLDG (IRWD OFFICE)
ZONE I
ZONE II
BONITA INTERTIE
TEMPORARY
M.W.D. EAST ORANGE CO. FEEDER NO 2
1
2
3
4
5

1 L. Parkview Lane
2 Arbor Lane
3 Paseo Picasso
4 Via Messina
5 Irvine Source

Figure 2. Sampling Location in the Irvine Ranch Water Distribution System

TABLE 1. SAMPLING LOCATIONS AND PIPE DESCRIPTION IN THE IRVINE WATER DISTRIBUTION SYSTEM

Site No.	Pipe Material	Location (Street)	Date Pipe was installed	Pipe Diameter (In.)
82	asbestos-cement	Solana	1975	8
53	asbestos-cement	Whitewood	1965	8
48	asbestos-cement	Dewberry	1965	6
13	asbestos-cement	Paseo Picasso	1973	8
8	polyvinylchloride	Via Messina	1970	4
25	asbestos-cement	Via Palatino	1970	8
117	asbestos-cement	L-Parkview Ln.	1970	4
67	asbestos-cement	Lancewood	1965	6
98	asbestos-cement	Birch Tree Ln.	1969	8
76	asbestos-cement	Arbor Lane	1973	8

TABLE 2. PLATE COUNT RESULTS (BACTERIA/ML) FROM THE IRVINE RANCH WATER DISTRIBUTION SYSTEM

		Media									
		Trypticase Soy Agar					Total Plate Count				
Street	Site	7/17[1]	8/8[1]	8/8[2]	8/23[1]	8/23[2]	7/17[1]	8/8[1]	8/8[2]	8/23[1]	8/23[2]
Whitewood	53	930	100	0	–	–	1330	200	185	–	–
Solana	82	200	–[3]	–	–	–	210	–	–	–	–
Dewberry	48	170	–	–	–	–	60	–	–	–	–
Paseo Picasso	13	0	1500	100	100	5	600	1100	0	100	10
Via Messina	8	300	200	100	5	0	300	110	65	35	0
Via Palatino	25	200	–	–	–	–	200	–	–	–	200
L. Parkview Ln	117	110	2300	900	100	10	1550	2100	165	300	10
Lancewood	98	40	–	–	–	–	40	–	–	–	–
Birch Tree Ln	76	40	–	–	–	10	30	–	–	150	–
Arbor Lane	67	40	270	200	35	–	180	690	50	–	5

[1] hydrant run 15 seconds before sampling
[2] hydrant run 1 minute before sampling
[3] not determined

TABLE 3. LOCATION OF SAMPLING SITES AND DESCRIPTION OF PIPE MATERIAL IN GARDEN GROVE WATER DISTRIBUTION SYSTEM

Site No.	Pipe Material	Location	Date of Pipe Installment	Pipe Dia. (In.)
1	galvanized	Sandra - beginning of street	1950's	4
2	galvanized	Sandra - end of street	1950's	4
3	asbestos-cement	Fairview - old main adjacent to new main	1953	-*
4	asbestos-cement	Fairview - new main adjacent to old main	1978	-
5	asbestos-cement	Dorada	1955	6
6	asbestos-cement	Norma Ln.	approx. 1954	4
7	asbestos-cement	Robert Ln.	1954	6
8	asbestos-cement	Kathy Ln.	1954	6
9	asbestos-cement	Morgan Ln.	1954	6
10	asbestos-cement	Harris St.	1973	8
11	asbestos-cement	Paladium	1959	8
12	asbestos-cement	Rockinghorse	1955	6
13	galvanized	Marshall Ln.	1940's	2

*Pipe diameter not known

TABLE 4. PLATE COUNT DATA (BACTERIA/ml) FOR GARDEN GROVE DISTRIBUTION SYSTEM WATER

Site	Media					
	Trypticase Soy Agar			Total Plate Count Agar		
	7/11[1]	8/7[1]	8/7[2]	7/11[1]	8/7[1]	8/7[2]
Sandra #2	TNTC	12100	100	TNTC	13900	40
Norma Ln.	160	200	0	TNTC	1000	0
Harris St.	3140	0	-[3]	TNTC	40	-
Robert Ln.	20	-	-	610	-	-
Paladium	TNTC	100	30	TNTC	80	50
Rockinghorse	660	100	20	TNTC	10	100

TNTC: too numerous to count

[1] hydrant run 15 seconds before sampling

[2] hydrant run 1 minute before sampling

[3] not determined

were sampled at monthly intervals from January through June 1979. Samples for bacteriological analyses were collected in 32 oz. sterile flint glass bottles. Sodium thiosulfate, 0.1 ml of 10% solution, was added to sample bottles prior to autoclaving. These samples were placed on ice and returned to the laboratory within 2 hours of collection. Samples for general mineral analysis, particle size, total organics and trihalomethanes were collected in designated containers. Samples to be analyzed for trace elements were placed in one liter plastic sample containers containing 4 ml of concentrated nitric acid.

Physical and Chemical Parameters

A number of physical and chemical parameters were assessed *in situ* during sampling (Figure 3): pH, temperature, dissolved oxygen, redox and residual chlorine; pH and redox were determined using an Orion Model 401 or a 497a specific ion meter. Temperature was determined using a standard centigrade thermometer or the temperature probe on a YSI dissolved oxygen meter model 51B. Dissolved oxygen was determined with a YSI model 51 dissolved oxygen meter. A Fisher-Porter portable amperometric titrator was used to determine free residual chlorine levels at all sites. Turbidity and electroconductivity were determined immediately upon return to the laboratory using HIAC N-610 Turbidimeter and YSI Conductivity Bridge model 31, respectively, using U.S.E.P.A. procedures.

Other physical and chemical analyses of the water were determined by J.M. Montgomery Consulting Engineers, Environmental Research Laboratory using U.S.E.P.A. procedures.

Particle Size

Particle size distribution determinations were performed at the laboratory. Particle size distribution was analyzed on a Hiac Model PC-320 particle size analyzer equipped with automatic bottle sampler and printer. Samples were taken in wide-mouthed bottles constructed of high quality glass. The bottles were thoroughly cleansed before use with detergent in a Bransonic 220 ultrasonicator for 20 minutes. This was followed by four rinses with particle-free deionized water which was produced by filtration through a 0.22 μm Nuclepore filter. Then the samples were rolled gently to ensure homogeneity prior to analysis. The particle-containing fluid was drawn through a particle sensor (see theory below) which counts the total number of particles in twelve size ranges covering from 2.5 to 150 μm. It was not deemed necessary to lower the size range to 1 micron. The Hiac particle counter functions on the principle of light blockage. (See Figure 4). Particles in fluid suspension flow through a channel past a window whose area is accurately known. A collimated beam of light shines through the liquid at right angles to the direction of flow. This light beam passes through the window and is received by a photoelectric detector (sensor). Whenever a particle traverses the light beam, a portion of the light is blocked from the sensor. This shadow, which represents the equivalent spherical diameter size range by calibration with standard mono-sized latex beads. Individual voltage drops, each corresponding to a particle, are summed on a twelve-channel digital display.

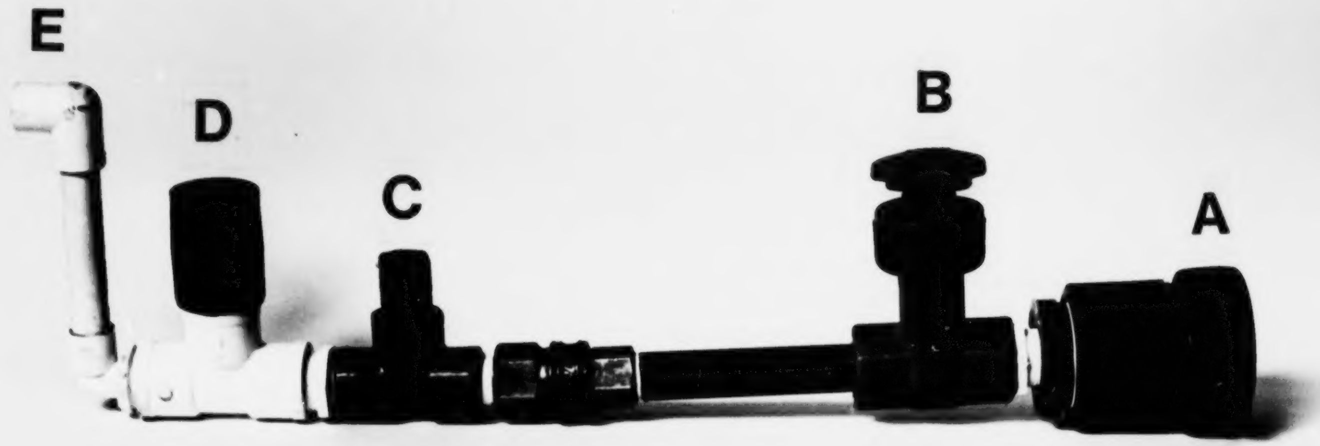


Figure 3. *In Situ* Hydrant Sampler for Physical and Chemical Measurements.

A Fire Hydrant Inlet, *B* Flow Control Valve, *C* Inlet for pH and Redox Probes, *D* Inlet for Dissolved Oxygen Probe and Temperature, *E* Outlet

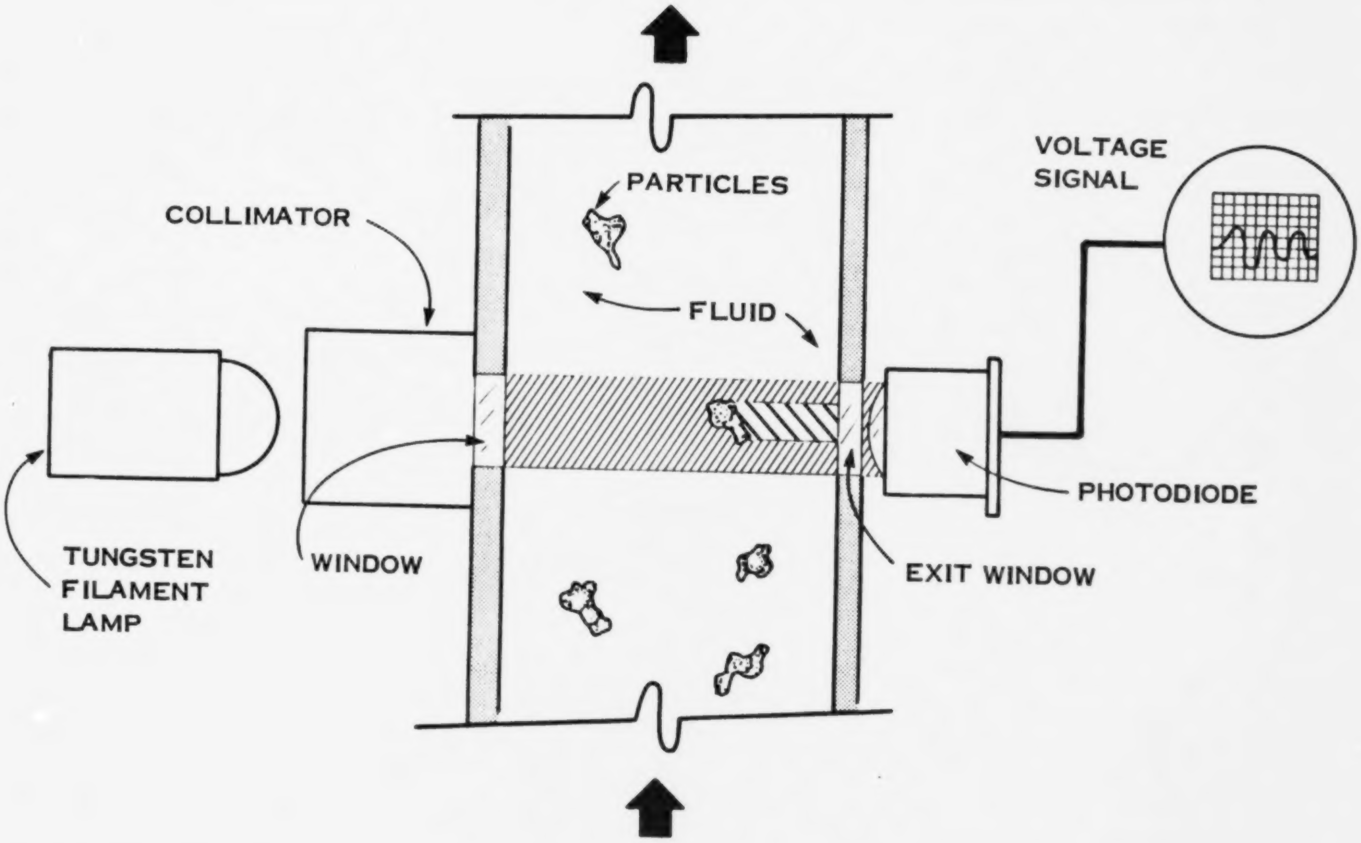

PARTICLES
VOLTAGE
SIGNAL
COLLIMATOR
FLUID
PHOTODIODE
TUNGSTEN
FILAMENT
LAMP
WINDOW
EXIT WINDOW

Figure 4. Principle of Operation for HIAC Particle Size Analyzer.

Chemical Characterization of the Water

Test	Method
Major cations	
Sodium	Flame photometer/Flame emission
Potassium	Flame photometer/Flame emission
Calcium	EDTA titration
Magnesium	EDTA titration then by difference
Major anions	
Bicarbonate, carbonate hydroxide	Titration
Chloride	Mercuric Nitrate Titration
Sulfate	Turbidimetric
Nitrate	Brucine colorimetric
Fluoride	Spadns colorimetric
Other analyses	
Silica	Colorimetric
Electroconductivity	Meter
Ammonia	Phenate colorimetric
Inorganic phosphate	Colorimetric ascorbic acid

The total dissolved solids (TDS) were analyzed by addition and Langelier Index was calculated.

Trace Metals

A Perkin-Elmer Model 305B atomic Absorption Spectrophotometer with a Model HGA 2200 graphite furnace, electrodeless discharge lamps and deuterium background correction were used for the trace metals work.

The following total metals as described in Methods for Chemical Analysis of Water and Wastes, U.S.E.P.A. were studied: iron, manganese, zinc, and cobalt.

Total Organic Carbon

Total organic carbon (TOC) was measured using a Dohrmann-Envirotech low-level analyzer (Model DC-54) capable of measuring purgeable organic carbon and non-purgeable TOC at concentrations as low as 10 and 50 mg, respectively. The DC-54 utilizes a UV-catalyzed phosphoric acid/potassium persulfate oxidation. Carbon dioxide from the oxidation is catalytically reduced to methane which is detected by a flame ionization detector. The detector response is integrated over time and the result is calibrated to read-out in micrograms of carbon per liter. A relative standard deviation of less than two percent is routinely attained.

Volatile Organics - Capillary Gas Chromatography

Purgeable Organics. The category of Purgeable Organics includes both Purgeable Halogenated Organics (PHO) and Purgeable Aromatic Hydrocarbons (PAH); the methodology of isolating these compounds from water is the same whereas their resolution, identification and quantification involves different techniques.

Purgeable Halogenated Organics. Many researchers have contributed to the dynamic headspace analysis of PHO. The dynamic headspace technique can be categorized into four basic components: purge-sorb, desorb, resolve, and identify-quantify.

1. Purge-sorb. Five milliliter samples were purged at ambient temperature (22°C). The purging efficiency of many compounds has been shown to decrease at sample volumes greater than one milliliter whereas five milliliters is sufficient to achieve detection limits of 1 μg/l for most compounds. Although improved partitioning of the organics to the gaseous phase can be achieved by elevating the temperature, this technique was not used as it may lead to falsely high values or degradation of certain organics. Increasing the ionic strength of the sample can also improve purging efficiency but since the organic compounds of interest to this survey all have high purging efficiencies, this salt addition was unnecessary.

   Various flow rates and purge times could have been selected to optimize recovery of different organics since this is dependent upon the retention indices of the sorbent trap and the partitioning efficiency from the aqueous phase. The water analyzed for this survey was purged for 12 minutes at a flow rate of 40 ml/min. with ultrapure helium. These conditions have been shown to have the best overall characteristics for the organic parameters of interest to this study and have been demonstrated to efficiently recover the organics listed in the scope of work. Higher flow rates realize no additional recovery. Lower flow rates can significantly reduce the recovery of certain organics with higher boiling points and larger dipole moments. Therefore, the flow rate of 40 ml/min. was maintained as a standard throughout the survey and was precisely controlled with a fine metering valve since small variations can lead to imprecision in some cases.

   Sorbent traps were carefully prepared using stainless steel columns (0.105" x 0.125") packed with 5 mm of silanized glass wool, 1 cm of 3 percent OV-1 on Chromosorb-W (60/80 mesh), 15 cm of Tenas GC (60/80 mesh), 8 cm of Grade 15 silica gel (35/60 mesh), and topped off with another 5 mm of silanized glass wool. The traps were carefully filled to prevent air pockets and non-uniformities which contribute to channeling effects.

2. Desorb. The sorbent trap was heated to 180°C in approximately 30 seconds and maintained at this temperature for 4 minutes. The absorbed compounds were then more favorably partitioned into the gaseous phase and 40 ml/min. of inert helium swept the compounds onto the front of the gas chromatographic column.

3. Resolve. The chromatographic separation of the mixture of organics isolated from the water samples was performed with a six foot coiled glass column (2mm x 0.25") containing 0.2 percent Carbowax 1500 on Carbopack-C (60/80 mesh). This primary analytical column was maintained at 60°C for three minutes then programmed to 160°C at 8°/min. The helium carrier gas was constantly maintained at 40 ml/min.

4. Identify-Quantify. The new fourth generation electrolytic conductivity detector is a bipolar pulse, differential electrolytic conductivity detector with improved stability, sensitivity, linearity and specificity. This detector consists of a microreactor and the differential conductivity cell. The microreactor provides more complete conversion of organics, especially brominated organics, to detectable species. Sensitivity has been lowered about an order of magnitude by the use of differential conductivity measurement which eliminates the high and variable background problems which have plagued PHO analysis with the conventional Hall detector. In addition the new cell geometry is 60 percent smaller and the band-spreading problems of the previous generation detector have been significantly reduced.

Although the discussion above has detailed the basic parameters of PHO analytical technology utilized, a more detailed step-by-step procedure is included below:

1. Blank Water. Blank water was prepared by taking water from a recirculating pure water system, filtering it through a glass column containing activated carbon and boiled if necessary. Blank water was not stored.

2. Glassware Cleanup. All glassware used for trace organics analysis were cleansed with routine laboratory soap, then rinsed with deionized water, analytical quality acetone and nanograde hexane. As a final step they were heated to 400°C (except volumetric and teflon containers) for two hours.

3. Standards. Standards of the PHO's were prepared gravimetrically by syringing or micropipetting the compounds into absolute methanol. The alcohol was added to the 25 ml volumetric flask to within a few mls of the fill line, stoppered and placed on an analytical balance. Without removing the flask from the scale nor directly handling the flask the organic standards were carefully added, ensuring that they directly dripped into the methanol. The weight of each addition was recorded, the volumetric flask was filled to volume, and the contents mixed to provide a homogeneous standard of known concentration. A secondary standard was made by adding a certain quantity of the primary standard with a volumetric pipette to a volumetric flask nearly filled with methanol. Aliquots of both the primary and secondary standards were sealed in glass ampules and refrigerated for future use (within two weeks).

Varying amounts of the secondary standard were spiked into one liter quantities of blank water with a volumetric pipette to provide daily standards at 1, 10, and 100 μg/l.

4. Sample Preparation. Sealed water samples were taken from cold storage and warmed up to room temperature (22°C) by exposure to laboratory ambient temperature or by heating in a water bath. The sample bottle was opened carefully and the sample poured into the barrel of a 5 ml syringe until it overflowed. The plunger was replaced and any air present was vented while the sample volume was adjusted to 5 ml.

5. Sample Introduction. The 5 ml syringe is attached to the 20 cm needle and the sample was injected into the purge chamber. One-half ml of an aqueous solution containing 10 mg/ml of the internal standard 2-Bromo-1-chloropropane and an orthophosphate buffer was then added by similar injection with a 1 ml glass syringe. The purge chamber was then sealed.

6. Sample Purge-sorb. A sorbent trap which had been conditioned for at least 15 minutes at 225°C with a continuous helium purge was attached to the purge device. (The trap conditioning step is important to remove memory effects from previous analyses, remove adsorbed contaminants, and expel water which degrades the performance of the silica gel.) The sample was purged for 12 minutes with 40 ml/min. of ultrapure helium and the organics were adsorbed in the trap which was held at ambient temperature. During this step, the gas chromatograph oven was cooled to ambient temperature and the GC column conditioned with the ultrapure carrier gas.

7. Desorb. The protruding GC needle of the desorb trap was placed through the injection septum of the gas chromatograph and the trap was rapidly heated to 180°C in about 30 seconds. This temperature was maintained for a total of 4 minutes to allow the organics to transfer to the front of the GC column. The trap was disconnected from the gas chromatograph and hooked into a separate trap conditioning apparatus that simultaneously conditioned two sorbent traps at 225°C.

8. Resolve-Identify-Quantify. At the end of the desorb step, the column oven was brought up to the initial operating temperature (60°C for the analytical column and 50°C for the confirmation column) and the temperature program of 8°/min. to 160°C was initiated after a 3 minute isothermal hold. Peak interpretation was performed by a HP 3385A Chromatographic Automation System which integrated peaks by continuous A/D conversion, performed internal standard calculations, automatically detected merged peaks, skimmed tangent peaks, plotted the gas chromatogram, and printed out a report in mg/l including sample identification, date and time of analysis.

9. Clean-up. During the Chromatographic run, the purging device and sample introduction syringe were rinsed twice with blank water. If high concentrations (>100 μg/l) or notable suspended solids were contained in the sample, the entire apparatus was cleaned according to the procedure outlined in 2. Glassware Clean-up.

Purgeable Aromatic Hydrocarbons (PAH). The analysis of Purgeable Aromatic Hydrocarbons is similar in many respects to the analysis of Purgeable Halogenated Organics.

1. Purge-sorb.

2. Desorb. These steps are exactly the same as for PHO's and will not be restated.

3. Resolve. The chromatographic separation of the mixture of organics isolated from the water samples was performed with a six-foot coiled glass column (2 mm x 6 mm) containing 5% SP 1200 + 5% Bentone 34 on Supelcoport (100/120 mesh). This primary analytical column was maintained at 65°C for 4 minutes, then programmed at 4°/min. to 170°C. The helium carrier gas was held at 40 ml/min.

   A second confirmation column had the same configuration, but was packed with 2% Carbowax 20 M on Porasil-C (80/100 mesh). This column was held isothermal for 3 minutes at 70°C and then programmed at 6°/min. to 180°C. The ultrapure helium carrier was constantly maintained at 40 ml/min.

4. Identify-Quantify. The Photo-Ionization Detector is highly sensitive to unsaturated organic compounds. Although its linearity range is $>10^2$, it is especially sensitive for benzene, toluene, and xylene.

1.	Blank Water.	
2.	Glassware Clean-up.	Same as procedures previously detailed for PHO analysis.
3.	Standards.	
4.	Sample Preparation.	
5.	Sample Introduction	Same as PHO analysis except internal standard is chlorotoluene.
6.	Sample Purge-sorb.	Same as procedures used for PHO analysis.
7.	Desorb.	
8.	Resolve-Identify-Quantify.	At the end of the desorb step, the column oven was brought up to the initial operating temperature, and held for four minutes prior to programming to 170°C at 4°/min. (The confirmation column was brought to 70°C and held isothermal for 3 minutes prior to programming to 180° at 6°/min.) Peak interpretation was performed by the HP Chromatographic Automation System.

9. Clean-up. This procedure was the same as that used for PHO analysis.

Total Microbial Enumeration Plate Count Procedures

Several media and two plating techniques were incorporated into the study to compare total count methodology. The media used and the corresponding plating technique (spread or pour plate) are shown in Table 5. All plating techniques were carried out in triplicate upon initiation of each method and later in duplicate. One milliliter, 0.1 ml and 0.01 ml of sample were inoculated. The incubation period for all techniques was at least 48 hours. Low nutrient R-2A medium was incubated for 96 hours. M-SPC medium incubations were extended beyond 48 hours to 96 hours on March 1, 1979. The formulations for low nutrient R-2A medium and M-SPC medium are shown in Table 6. As shown in Table 5 trypticase soy agar and Mueller-Hinton media were discontinued during the initial phase of the study due to low plate count results.

Pigmented Count: Total Count Ratios

Ratios of pigmented bacteria to the total bacterial counts were obtained for all sites beginning in October, 1978. Pigmented colonies were considered to be all those in which colors and shades other than white or cream-white were noted. There were some instances where clear-cut decisions were impossible. However, the vast majority of decisions were based on clear demarcations. The ratios are expressed as the percentage of pigmented bacteria in the total bacterial count (pigmented and non-pigmented types).

Bacterial Identification

Bacteria isolated from Paseo Picasso in Irvine and Rockinghorse in Garden Grove distribution systems were identified at quarterly intervals from September, 1978 through April, 1980. Bacteria were isolated and identified from source water from January, 1979 through April, 1980. All the bacteria present on total plate count media, spread plates incubated at 35°C, were selected for identification at the above noted times.

Approximately 100 bacteria were identified at each test location. These organisms were purified by restreaking onto total plate count (TPC) agar. Organisms were stored at 4°C on TPC agar until time of identification. Twenty-four hour cultures of bacteria were gram stained and screened through a modification of the Lassen method (Table 7).

Modification of Bacterial Identification Procedures

As of October, 1979, all gram negative rod isolates were identified according to the procedure developed by the Lassen Method (72). Any isolate preliminarily identified as Acinetobacter, Alcaligenes, Flavobacterium/Moraxella or Pseudomonas was subjected to further testing to determine its respective oxidative-fermentative ability. Isolates are streaked and stabbed into four tubes of O-F Basal Medium (Difco) each containing 1% of one of the following carbohydrates: glucose, dulcitol, saccharose, or lactose.

TABLE 5. MEDIA AND PLATING TECHNIQUE EMPLOYED IN TOTAL BACTERIAL STUDIES

date initiated	date ended	Medium	Source	Technique	Incubation Temperature
11-9-78	4-28-80	Total plate count (TPC)	Difco	spread plate	35°C
6-28-78	4-28-80	TPC	Difco	pour plate	35°C
1-2-79	4-28-80	TPC	Difco	spread plate	35°C
1-2-79	10-79	TPC	Difco	pour plate	20°C
9-13-79	10-12-79	Mueller-Hinton	Difco	spread plate	35°C
6-28-79	10-12-79	Trypticase soy agar	BBL	spread plate	35°C
3-1-79	4-28-80	m-SPC medium	special (121)	membrane filter	35°C
1-2-79	4-28-80	Low nutrient R-2A	special (101)	spread plate	20°C

TABLE 6. FORMULATION OF M-STANDARD PLATE COUNT AND LOW NUTRIENT-R2A MEDIA

m-SPC

grams/100 ml distilled water

Peptone	2.0
Gelatin	2.5
Glycerol	1.0
Agar	1.5

Before adding glycerol, adjust pH to 7.1, if necessary, with in NaOH, then heat to dissolve ingredients. Add glycerol, autoclave 5 min at 121°C.

Low Nutrient R-2A Medium

grams/l distilled water

yeast extract	0.5
proteose peptone no. 3	0.5
vitamin free casamino acids	0.5
glucose	0.5
soluble starch	0.5
K_2HPO_4	0.3
$MgSO_4.7H_2O$	0.05
Sodium pyruvate	0.3
agar	20.0

Adjust pH to 7.2 with crystalline K_2HPO_4 or KH_2PO_4. Sterilize at 121°C for 15 min.

TABLE 7. BACTERIAL IDENTIFICATION PROCEDURES

I. Bacterial Identification Techniques for Gram Negative Bacteria

A. Gram Stain

B. Lassen Method
Reference: Lassen, Jorgen (1975) "Rapid identification of gram-negative rods using a three-tube method combined with a dichotomic key. Acta path. microbiol. scand. Sect. B, 83: 525-533

Tube I: Combined lactose-glucose-H_2S tube demonstrates
1. fermentation of lactose
2. fermentation of glucose and gas formation
3. H_2S production
4. lysine decarboxylase
5. culture growth on this tube may be used to perform oxidase test

Tube II: Mannitol-motility tube demonstrates
1. fermentation of mannitol
2. motility
3. nirate reduction

Tube III: Urea-Indole tube demonstrates
1. urease
2. indol production
3. tryptophan deaminase

B. Kligler Iron Agar (Difco) used in conjunction with Lassen Method demonstrates:
1. fermentation of lactose
2. fermentation of glucose and gas formation
3. H_2S production

Kligler Iron Agar is a richer medium tube I of the Lassen Method therefore producing better growth of the culture.

D. Additional tests to be used for further genus differentiation of Gram Negative Bacteria

1. Salmonella/Serratia groups
   test to be performed: DNase production
   a) Salmonella- DNase not produced
   b) Serratia- DNase produced

(continued)

TABLE 7 (continued)

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2. Flavobacterium/Moraxella groups
   test to be performed: Flagellar stain (reference to follow)
   - a) Flavobacterium-possible flagella - also pigmentation
   - b) Moraxella - no flagella. non-pigmented

3. Acinetobacter/Moraxella groups
   test to be performed: Oxidase - penicillin
   - a) Moraxella - oxidase (+), sensitive to penicillin
   - b) Acinetobacter - oxidase (-), resistant to penicillin

4. Pseudomonas/Alcaligenes groups
   test to be performed: flagellar stain
   - a) Pseudomonas - polar flagella
   - b) Alcaligenes - peritrichous flagella 1-4 flagella

II. Classification of Gram-positive Bacteria (Bergey's Manual)

Actinomycetes

1. gram positive
2. branched filaments w/granular surface (powdery)
3. colors vary: white, opaque, cream
4. some very irregular shapes - (piling type of growth)
5. musty odors produced.
6. non-motile

Streptomyces

1. gram positive
2. spores produced
3. wide variety of pigments: white, gray, yellow, pink

Coliform Enumeration

Detection of coliforms was carried out using both membrane filter and the 5 tube Most Probable Number (MPN) techniques described in Standard Methods: Sections 909A and 908A. Inoculum volumes were 200, 100, 50 and 25 ml for the membrane filter method and 5, 10 ml tubes for the MPN method. Confirmation of colonies appearing on m-endo medium began in November of 1978. All m-endo coliform data reported prior to this time was not confirmed and is so designated. All colonies which appeared as typical coliforms were confirmed by inoculation into lauryl-tryptose medium (Difco). M-FC technique was used to test for fecal coliforms. Identical volumes to the m-endo procedure were filtered.

In addition to the Standard Methods MPN tests, the MPN method was modified by transferring all tubes which showed growth but no gas production during the presumptive portion of the test to confirmatory and elevated temperature media.

Coliform Identification

All confirming coliforms from m-endo medium were identified beginning in May, 1979 using either the Lassen Method or API-20 enteric system. Sporadic identifications were carried out prior to May. Also, some tubes which were positive for the confirmed section of the coliform test were screened for identification. Media from BGLB positive tubes were streaked onto EMB agar and the isolates were screened for identification using the aforementioned methods. All false-negatives have been isolated and identified since the initiation of the study in July, 1978.

Gram positive organisms were identified utilizing the methods in Bergey's Manual (18) section for actinomycetes (Table 7).

Fungal Enumeration

Fungi were enumerated at all sites in both systems six times during the first year of the investigation, twice during the winter (January 2 and 18, 1979), spring (April 12 and 26, 1979) and summer (July 19 and August 2, 1979). Twenty ml of distribution water was filtered through a 0.45 µm membrane filter. Five replicate samples were made for each test sample. The membrane filters were placed on Czapek-dox agar, except on January 18, when Sabouraud Dextrose and Sabouraud Maltose agars were used. The incubation temperature was 25°C and filters were enumerated under the dissecting microscope after 4 and 10 days incubation. Fungal colonies were isolated onto Czapek-dox agar and stored for later identification. The frequency of fungal colonies per 100 ml was calculated. Numbers of fungal colonies were recorded and numbers of fungal species estimated.

Coliform Suppression Experiments

In order to assess the extent and significance of bacteriocin-like substance (BLS) production by non-coliform bacteria in water distribution systems, bacterial isolates from a chlorinated and non-chlorinated water distribution system were screened for their ability to inhibit the growth of three members of the coliform group: *E. coli*, *Klebsiella* sp. and *Enterobacter aerogenes*. In addition, bacterial isolates from positive MPN presumptive medium and false-negative tubes, and from positive confirmatory tubes, were identified and screened for their ability to inhibit the predominant coliform isolated from the same tubes.

Prior to initiating the coliform suppression experiments (bacteriocin experiments) as just described, a series of experiments were designed to obtain information concerning the potential ability of water distribution system isolates to act as suppressors of coliform growth and importantly, gas production, in the standard MPN method. A rapid screening procedure for coliform suppressors is desirable to clarify the ecological role of bacteria considered competitor/antagonists to *Escherichia coli* and other coliform bacteria in water distribution systems. Six different experimental formats were examined to assess inhibition/suppression of coliform organisms by non-coliform organisms. Each method and its results are discussed briefly in Table 8.

(i) Test Format I - Overlay

The classic method (40) for testing bacterial sensitivity to bacteriocin production by another organism was used by Fredericq (40) on a total of 20 isolates. The test organisms under investigation were grown in TPC broth at 35.5°C for 24 h. Ten µl of the broth culture were inoculated onto TPC plates, each plate accomodating 5-7 test organisms. These plates were then incubated at 35.5°C for 24 h. After 24 h each plate was inverted over a CCH_4 source for 2 h followed by 3 ml overlay of total plate count agar (0.5% agar) containing approximately 10^5 *E. coli*/ml. The plates were then reincubated for an additional 24 h at 35.5°C. Zones of *E. coli* inhibition appeared in the overlay above test organisms that produce a colicin. It is important to emphasize the sensitivity requirement for zones of inhibition to be observed. A control strain of *E. coli* (ATCC #14763) was obtained and produces colicin V. Because an individual strain of *E. coli* produces no zone of inhibition, one cannot rule out the possibility that it is resistant to suppression, only that it is resistant to the colicin (if any) that the test organism produced.

(ii) Test Format II - Spot Culture

In this method, 10 µl of a 24 h TPC culture of *Escherichia coli* were spread onto a TPC agar plate. Followed by the addition of 0.01 ml of potential antagonist organisms which had been incubated at 35°C for 24 h TPC broth cultures. The plates were then incubated at room temperature for 24 h and zones of *E. coli* inhibition around the test organisms were recorded.

(iii) Test Format III - Cross Streak

The cross streaking of potential bacteriocin producers and their counterpart sensitive strains was attempted as a rapid screening method. This method is similar to the 90° streak method discussed by Reeves (102, 103). The difference lies in the continuation of the streak through the bacteriocin producer. Inhibition was judged to occur when zones of no-growth adjacent to the bacteriocin producer were observed. No instances of inhibition were recorded among the 25 organisms tested. However, 12 instances of organisms growing in a "pinched-in manner" were observed. Interestingly, when *E. coli* (ATCC #1512) is cross streaked with itself the same pinched-in growth at the point of crossing occurred. This may be due to rapid absorption of diffusible nutrients at the agar surface. As a result the bacteria at the edges near the center of the cross streak cannot spread outward as rapidly as those cells at the ends of each cross streak. The net result is a growth pattern resembling two crossed dumbbells.

(iv) Test Format IV - Cross-Streak Overlay

The cross-streak overlay method, a combination of Formats 1 and 3, was tried for bacteriocin detection. Twenty-five bacterial isolate streaks were obtained as in Format 3 and overlaid with TPC agar (0.6% agar) with 10^6 *E. coli*/ml of spiked TPC, incubated at 35°C for 24 h and then zones of inhibition in the agar were measured. No instances of inhibition were noted.

(v) Test Format V - Edge Streak

Test organisms were incubated 24 h at room temperature. A 1 mm loopful of the test organism culture was streaked across TPC agar plates and incubated 24 h at room temperature. At 24 h, a 1 mm loopful of a 24 h culture of *E. coli* was streaked perpendicular to the grown test organism without crossing the streaks and the plate incubated for an additional 24 h at 35°C. At this time the plates were scored for suppression of *E. coli* growth by the test organisms. A positive test was recorded when the edge of the *E. coli* streak failed to grow to the edge of the test organism. Ten organisms were selected from the previous formats which appeared to be inhibitory. Of these ten, 7 caused the *E. coli* streak to spread out farther as distance increased from the perpendicular test organism streak. In three instances *E. coli* grew up to the test organism. A control experiment in which *E. coli* was streaked perpendicular to itself produced results similar to that seen in the cross streak (Format 3) control. That is, the *E. coli* spread out farther as distance from itself increased. This suggests a nutrient competition phenomenon occurred at the agar surface, but further study would be required to confirm this.

(vi) Test Format VI - Membrane Filter Overlay

This method involved the use of membrane filters to assess the interaction between *E. coli* cells on the surface of the filter and the test organisms on the surface of a TPC agar plate. The test organisms were grown in TPC broth at 35.0°C for 24 h. Ten milliliters of the broth culture were spread onto a TPC plate. At this time 1 ml of a 5 mM phosphate buffer solution containing approximately 30 *E. coli* cells per ml were filtered through a 0.2 μm Nuclepore filter. The filter was then placed on the surface of a TPC plate that had been spread with the test organism and incubated at 35.5°C for 24 h. The filters were then examined for *E. coli* colonies and the growth results compared to *E. coli* growth on control plates (no test organisms applied) variations of this method using different media and filter brands were examined.

Initial results were promising. The 41 cultures tested as *E. coli* suppressors produced filter counts which followed a normal distribution. Both size and number of *E. coli* colonies varied widely and depended on the test organisms spread on the plate. Four organisms totally suppressed *E. coli* growth on the filter. Of the four organisms, one was *E. coli* (ATCC #14763), an *E. coli* colicin V producer, and two were *Klebsiella* sp.

A problem arose, however, when the *E. coli* organism would not grow on a filter placed on a TPC plate spread with "itself." The failure of the organism to "grow on itself" seriously limits the usefulness of the method. It also suggests that suppression of the growth of *E. coli* by the four test organisms was due to the release of an inhibitory substance.

Experiments from Format VI suggested that the presence of certain bacterial cells beneath the filter can prevent or restrict the growth of the *E. coli* organism on the membrane surface. The nature of the apparent suppression could be explained on the basis of mechanical blockage of the nutrient flow through the membrane or rapid utilization of all available nutrients by the underlying test organisms. Regardless of the cause, this drawback prevents the use of this method as a screening procedure for colicin or other inhibitory substance producing bacteria.

Of note was the greater recovery of *E. coli* on filters placed on Brain Heart Infusion agar (Difco) compared to PC agar (Difco) or Nutrient agar (Difco), suggesting nutrient competition could be extremely important. Further, when filters without visible growth or that showed total suppression of *E. coli* growth were placed into presumptive MPN medium, all produced gas within 24 h at 35.0°C.

Isolation of bacteria for the bacteriocin experiments. The two distribution systems were sampled form January, 1979 through September, 1979. A total of 2,215 isolates were obtained during January, April, May, July, and September. At each sampling time approximately 100 bacteria from each of six sites in the systems were isolated from PC agar spread plates. Sampling protocol was identical to that appearing under bacterial identification (72). Plates selected for bacterial isolation had been spread with 0.1 ml of sample and incubated at 35°C for 48 h. The isolates were streaked onto PC agar, isolated colonies transferred to PC agar slants, and the slants stored at 4°C until identification.

Isolates. The isolates were screened according to the method of Fredericq (40) with minor modifications. Tubes containing 3 ml PC broth were inoculated with the culture and incubated for 4 days at room temperature. The tubes exhibiting growth were vortexed and 0.01 ml of each broth culture spotted onto PC agar plates. Each broth culture was spotted onto three separate plates to accomodate an agar overlay. Six to nine organisms were placed on each plate using each of three coliforms. The plates were then incubated for 48 h at room temperature. In a fume hood, the 48 h plates were inverted over a plastic petri dish containing a standard glass microscope slide upon which several drops of reagent grade chloroform (Mallinckrodt) had been placed. This procedure simplified Bauernfeind and Burrow's (10) modification of Gillies and Govan's (47) method. After the isolates had been exposed for 30 minutes, additional drops of chloroform were placed on the slide. The total exposure was 1.5 h.

Following removal from the chloroform exposure, each of the three replicate plates was overlaid with 3 ml of 0.6% agar plate count meduim containing 0.01 ml of a 24 h PC broth culture of E. coli, Klebsiella sp. or Enterobacter aerogenes. The overlaid plates were incubated for 24 h at 35°C, examined, and inhibition zone diameter (mm) in the coliform lawn adjacent to the test isolate was measured.

Analysis of positive and false-negative presumptive MPN tubes. A positive and a false-negative presumptive MPN tube, both of which had confirmed in Brilliant Green Bile (BGB, Difco) as coliforms, were selected for further study. Eosin Methylene Blue agar (EMB, Difco) plates were streaked with 0.01 ml of medium from the respective BGB tubes and incubated for 24 h at 35°C. Examination of the EMB plates revealed non-typical pink mucoid colonies from both tubes. Representative colonies were then purified and identified as Enterobacter cloacae using the Lassen method (72). These isolates were then inoculated into Lauryl Tryptose Broth (LTB, Difco) and incubated for 24 h at 35°C. Both isolates produced acid and gas reactions typical of members of the coliform group.

The positive LTB tube, and its corresponding BGB tube, together with the false-negative tube and its corresponding BGB tube were serially-diluted in phosphate buffer (118) and the dilutions plated onto PC agar to yield approximately 50 colonies per plate. All colonies from a given plate were restreaked on PC agar, placed on PC agar slants and identified using the Lassen Method (72).

After identification, the isolates were tested for ability to produce BLS specific against the associated predominant coliform isolated from the corresponding MPN tube using the method of Frederiqc described earlier. An additional modification was required for extremely mucoid colony types (primarily Aeromonas hydrophila) which were resistant to chloroform inactivation. Bacterial growth, after soft agar overlay, prevented zone measurements. To circumvent this problem, the colonies were carefully scraped off the agar surface using sterile spatulas prior to chloroform exposure. This effectively reduced the overgrowth problem yet left undisturbed any BLS which had diffused into the agar. The previously described overlay procedure was followed to complete the screening.

Collection and Preparation of Pipe Samples for Scanning Electron Microscopy

A two meter segment of galvanized iron water main (10 cm diameter) was removed intact during routine maintanence operations from the Garden Grove, Calfiornia municipal water distribution system (Lampson Ave.). The City of Garden Grove is normally supplied by unchlorinated ground water. However, during the summer months, when demand is especially high, the city purchases 10-15% of its water from the Metropolitan Water District of Southern California. The latter is fully treated, chlorinated, surface water consisting of a blend of Northern California and Colorado project water. This particular section of pipe had been in continuous service for approximately 25 years. The open ends of the pipe were sealed with Saran Wrap to prevent contamination and dehydration of the pipe interior during transit to the laboratory. At the laboratory, the pipe was cut in half and inspected visually revealing the presence of a hard, brittle substance (about 0.5 cm thick), black in color, and presumed to be concrete or porcelain, which lined the interior of the pipe. Several small fragments of this material were chipped from the inside of the pipe and immersed in an ice-cold solution of 2.5% (w/v) EM-grade glutaraldehyde in 50 mM monobasic potassium phosphate buffer, pH 7.0. Following overnight fixation at 4°C, the pipe fragments were gently washed in distilled water, dehydrated in an increasing ethanol concentration series (30, 50, 75, 85, 95, 100% x 3) and then transferred to "Freon 113" prior to critical point drying according to Cohen et al. (26). The specimens were mounted on aluminum stubs with conducting silver paint, coated with gold/palladium (60:40), and examined in a Hitachi model SU 500 scanning electron microscope operated at an accelerating voltage of 15 kev and 10 mm working distance. Pipe samples to be examined by x-ray energy dispersive microanalysis were mounted on stubs with collodial graphite and coated with a layer of evaported carbon. Black and white photographs were made on Polaroid N/P55 film.

Collection and Preparation of Suspended Particles for Scanning Electron Microscopy

Water samples were collected from selected fire hydrants or well outlets in clean, sterile, flint-glass bottles to which 0.1 ml of 10% (w/v) sodium thiosulfate was added prior to autoclaving. Before sampling, the hydrant was flushed for one minute at a flow rate of approximately 800 liters per minute. Suspended particulate matter was recovered from water samples by filtering 20 to 50 ml aliquots through a 0.2 μm pore size Nuclepore membrane filter (Nuclepore Corporation, Pleasanton, California). The particles captured on the membrane surface were immediately overlaid with 20 ml of the 2.5% glutaraldehyde fixative solution. After fixation at 23°C for 30 minutes, the glutaraldehyde was removed by filtration and the membrane washed twice by filtration in pre-filtered distilled water. The membrane was dehydrated in ethanol and "Freon 113" as described above; critical point drying was avoided to prevent loss of particulate material from the surface of the membrane. The membrane was rapidly air-dried directly from the absolute "Freon 113", cut into 1-cm circles, and mounted on the aluminum stubs. The stubs were coated with gold/palladium (60:40) prior to scanning electron microscopy.

X-ray Energy Dispersive Microanalysis

The carbon-coated specimens were examined in a Cambridge model S4 scanning electron microscope operated at 25 kev which was equipped with an x-ray energy dispersive microanalysis unit (Ortec, DELPHI). A continuous x-ray energy spectrum from 0 to 10 kev was integrated over a minimum of 200 seconds for each elemental scan. The K_α and K_β energy lines were utilized for identifying the elements present in the sample. The limit of detection of any particular element in the sample was about 1500 ml/l. Control elemental scans were performed on background areas (e.g., the naked membrane surface) and on adjacent areas of the cell surface to eliminate possible artifacts introduced by topographical electron scattering. Quantification was achieved by interfacing a PDP-11 computer with the x-ray microanalysis multichannel processor.

Ratios of the corrected areas under the peaks for the elements of interest were automatically computed and normalized to the amount of iron in the specimen.

Elemental analyses of a total of 693 carbon-coated particles ranging in size from 2.0 to 50.0 μm in diameter were performed using an ETEC Autoscan scanning electron microscope equipped with a KVEX x-ray detector (model 5100) multichannel analyzer interfaced with a Le Mont Scientific B10-E image analysis system. Operating conditions for the latter analyses were: 25 mm distance, zero degrees tilt, and 20 kev excitation energy.

Measurement of Bacterial Chlorine Resistance:

(i) Disc assay procedure. The disc assay technique is similar in principle and implementation to the Kirby-Bauer antibiotic disc assay method (9). Bacteria which had been previously isolated from the two water distribution systems described above, and stored refrigerated on agar slants for periods up to five months, were inoculated into disc assay broth (containing per liter of distilled water): tryptone, 0.5 g (Difco); yeast extract, 0.5 g (Difco); and glucose, 0.5 g (Difco). Cells were incubated for 24 hours in an orbital shaker at 23°C at which time each culture was diluted in fresh disc assay broth to a barely visible turbidity. Twenty microliters of the diluted bacterial suspension was spread plated on the same medium solidified with 15.0 g of agar (Difco) per liter. Three plates of an environmental strain *Escherichia coli* (strain #40 of our culture collection) were routinely prepared with each group of isolates tested in order to control for internal experimental variation.

Sterile filter paper discs (7.0 mm in diameter) of Whatman #42 ashless paper were impregnated with 8.0 ul of solutions of sodium hypochlorite (either 1250, 2500, or 5000 mg/l) in 10.0 mM potassium phosphate buffer, pH 7.0. Each disc was placed on the freshly seeded lawn of the test bacterium and the plate incubated at 23°C for 48 hours. The diameters of the clear zones of growth inhibition which developed surrounding the discs were measured and recorded.

(ii) Membrane filtration assay procedure. All operations described below were carried out aseptically at room temperature (23°C) and all reagents were prepared using ultrapore glass distilled water.

An aliquot of the water sample (usually 0.1, 1.0, or 10.0 ml, depending on bacterial density) was filtered through a 0.2 μm pore-size Nuclepore membrane filter (Nuclepore Corp., Pleasanton, CA) held in a standard 47-mm diameter glass Millipore filter assembly (Millipore Corp.). The cells captured on the membrane surface were immediately washed with 10.0 ml of 5.0 mM potassium phosphate buffer, pH 7.0. After the buffer passed through the membrane filter, the vacuum was broken and the membrane rapidly flooded with 20.0 ml of a 0.1 mg/l solution of sodium hypochlorite. The chlorine solution was prepared by diluting a stock 5.0% (w/v) aqueous solution of sodium hypochlorite (Baker Analyzed Reagent, J.T. Baker Co.) in 5.0 mM potassium phosphate buffer, pH 7.0. A sterile Petri-dish lid was placed over the filter assembly and the sodium hypochlorite solution was allowed to remain in contact with the membrane surface for 2.0 minutes. At the end of 2.0 minutes the vacuum was reapplied.

Immediately after the chlorine solution passed through the membrane (approximately 15 seconds), the cells were washed with 10.0 ml of a 10.0 mM solution of sodium thiosulfate in the 5.0 mM potassium phosphate buffer. The membrane was removed from the filter apparatus under vacuum and placed on the surface of solid m-SPC medium. Duplicate membranes were prepared as above for several chlorine concentrations (e.g., 0.05, 0.1, 0.5, 1.0, and 10.0 mg sodium hypochlorite/l) and bacterial densities (0.1, 1.0, and 10.0 ml of water sample filtered). Several untreated control membranes exposed only to the phosphate buffer were prepared along with the chlorine-treated membranes. All plates were incubated at 35°C and the colonies which arose were enumerated after 48 and 96 hours. The number of bacterial survivors enumerated on chlorine-treated membranes were expressed as a percentage of the bacterial colonies on the untreated control.

(iii) Liquid assay procedures. Bacteria were grown in Trypticase Soy Broth (TSB, Difco) overnight (at 18 hours) on an orbital shaker at 23°C. The cells were harvested by centrifugation (3500 x g, 15 minutes) and washed twice in sterile ice-cold 50.0 mM potassium phosphate buffer, pH 7.0. The washed bacteria were resuspended in buffer to an optical density of 1.05 (Hitachi model 100-20 spectrophotometer) and 10.0 μl of this suspension was distributed into each of twelve reaction tubes containing 10.0 ml of the 50.0 mM phosphate buffer. The final cell concentration in each tube was approximately 4 x 10^6 per ml.

Each tube was mixed thoroughly and at t = 0 minutes a different amount of sodium hypochlorite was rapidly injected (with continuous mixing) into eleven of the tubes. The last tube served as an untreated control and received no chlorine. The applied sodium hypochlorite concentrations in the tubes was varied from 0 to 2.5 mg/l. After ten minutes at 23°C, 100 μl of sterile 1.0 M sodium thiosulfate was added to each tube. The tubes were chilled to 2°C and aliquots of the test solutions were plated on Total Plate Count agar (Difco) to recover surviving bacteria. The plates were incubated at 35°C for 48 hours at which time colonies were enumerated.

When the effect of organically-combined chlorine was to be examined, the bacterial isolates were grown in disc assay broth and diluted without prior washing to the same optical density in fresh broth yielding a final concentration of dissolved organic substances of 1.5 mg/l after dilution in the 10.0 ml reaction tubes. This concentration of organics combined with all available chlorine added.

For kinetic studies, E. coli cells were grown and washed in buffer as described above and diluted into 500 ml of sterile 10.0 mM potassium phosphate buffer, pH 7.0, yielding a final cell concentration of approximately 1.0 x 10^7 bacteria per ml. To initiate the reaction, sodium hypochlorite was added with continuous stirring to give a final concentration of 0.1 mg/l. One-tenth ml was removed at intervals and injected into 10.0 ml of ice-cold potassium phosphate buffer containing 10.0 mM sodium thiosulfate and 0.1% (w/v) peptone (Difco) to stop the reaction. The cells were subsequently diluted and plated on Plate Count agar (Difco) and survivors counted after incubation for 48 hours at 35°C.

Chlorine demand measurements. The chlorine demands of polycarbonate Nuclepore membranes and nitrocellulose Gelman membranes were determined by immersing a single membrane (47-mm in diameter) in 200 ml of a sodium hypochlorite solution in 5.0 mM potassium phosphate buffer, pH 7.0. Fourteen separate chlorine solutions ranging in concentration from 0 to 2.5 mg of sodium hypochlorite/l were examined for each of the membranes for 45 minutes at 23°C. The remaining free chlorine residual was determined amperometrically using a Fischer-Porter chlorine titrator (model 17T1010). The chlorine titrations were carried out according to the manufacturer's instructions using phenylarsine oxide as the reductant. The chlorine demand of the membrane was that concentration of applied chlorine that caused a sharp upward inflection in the titration curve, followed by a linear increase in the measured free chlorine residual.

The chlorine demand of the disc assay broth was determined by two methods. The first consisted of dosing a series of 15-ml aliquots of the medium with increasing amounts of a 5.0% (w/v) sodium hypochlorite solution. After a one-hour holding time, the total residual chlorine was determined using the acidic iodine-thiosulfate procedure with starch endpoint.

The second method involved amperometric titration of both free and combined residual chlorine. The medium was diluted 1/100 and several 200-ml aliquots were dosed with a 5.0% (w/v) sodium hypochlorite solution. Following a 30-minute contact time the chlorinated solutions were titrated with phenylarsine oxide to an amperometric "dead-stop" endpoint at pH 7.0 for free chlorine, at pH 7.0 with added iodide for monochloramine, or at pH 4.0 with added iodide for dichloramine. The total chlorine residual was calculated by summation of the endpoints.

SECTION 5

RESULTS AND DISCUSSION

SANITARY SYSTEM EVALUATION

Description of Garden Grove System

The primary source of ground water is from Well No. 6321, with peak requirements supplemented by Well No. 6064. Well No. 6321 has a rated capacity of 2300 gpm. It discharges into two 10 million gallon reservoirs and then is boosted into the distribution system. The two reservoirs are underground, reinforced concrete structures. No chlorination facilities are provided.

Well No. 6321 was drilled in 1969 to a depth of 1158 feet with the first perforation at 440 feet. Several impervious clay layers are present above the first perforation. In 1978, the well produced 1145 acre feet of water. By contrast, Well No. 6064 discharges through a hydropneumatic tank to the distribution system. The well is used as a standby unit. The well was drilled by a private water company in 1954 and purchased by the city in about 1956. The depth of the well is 454 feet with the first perforation at 417 feet. Over 160 feet of impervious strata are found above the first perforation. Well No. 6064 has a capacity of 450 gpm, with a total 1978 water production of 445.5 acre/ feet.

Both wells are located over 50 feet from any sewer lines, the current spacing criteria used by the California State Department of Health services (State Health Department) in the evaluation of potential sanitary hazards.

A field inspection of both wells was performed to determine if the wells had any defects in the construction that could impact the bacteriological quality of the supply. The field inspection revealed the following:

1. Well No. 6321 is well maintained. No defects that had sanitary significance were noticed in the construction of Well No. 6321.
2. Well No. 6064, a standby facility, was not as well maintained. In addition, the following defects were noticed.
    a. Drainline on tank terminated below ground. Possible source for contamination if the area is flooded and negative pressure is experienced in the tank.

    b. The air vacuum relief valve outlet is not screened and the valve leaks. The purpose of the valve is to evacuate air from the well casing upstream from the well check valve during well start-up. Also, it allows the drainage of the water column upstream of the check valve during well shut off. During well start-up, the valve exhausts air to the atmosphere and during well shut down it allows air into the discharge piping and well casing. During the well shut off operation, if the inlet of the valve is submerged, liquid will be pulled into the well casing causing a potential contamination of the well.

c. Hydropneumatic tank is not level but slopes. This condition could have occurred due to differential settling at the well site area. If this is the case, further settling could cause the well discharge line to rupture.

Water Quality

Evaluation of water qualtiy data from both wells indicate:

1. Both wells produce water of good physical and chemical quality as determined by the California State Department of Health Services and U.S. EPA drinking standards.

2. No positive coliform samples were found in special bacterial sampling programs of wells.

3. A monthly nitrate monitoring program of well supplies in 1975-76 revealed that both Well Nos. 6321 and 6064 did produce water with nitrate concentration less than 30 mg/l as NO_3.

4. Routine distribution system coliform monitoring of the study area indicated no repeat coliform positive samples. In almost all cases, samples were negative for coliforms.

5. Physical quality monitoring in the distribution system revealed color values less than 5 units, and threshold odor less than 1. Turbidity values in the distribution system are generally in the range of between 0.5 and 1.0 T.U.

Water System Operational Procedure

The City of Garden Grove has no regular water main flushing program; flushing is performed on an "as needed" basis. In addition, there is no policy for the disinfection of existing mains. The city follows the AWWA 6601 policy on the disinfection of new water lines. In terms of new water main construction, the city follows the state-approved policy on sewerline/water main separation. Records show no deviation from this policy. No low pressure (less than 5 psi) or gravity mains are present in the system.

The city maintains a record of all consumer complaints relating to the water system. In the study area, no such complaints were made that are of a water quality nature. In addition, cross connection control devices in the study area are inspected and tested periodically (see attached list).

Description of Irvine Ranch Water District (IRWD)

The study area consisted of the southern portion of the IRWD service area as indicated by the attached system map (Figure 1).

System Description

IRWD receives over 90 percent of its domestic water from the Metropolitan Water District of Southern California. The study area is served through MWD connection OC-39. The connection is located on the East Orange County Feeder No. 2 and has a capacity of 40 cfs. Essentially, the connection consists of a metering device located in a vault structure. The water received through the connection is a blend of Colorado River and State Project waters that is treated at the Diemer Water Treatment Plant and stored in the San Joaquin Reservoir. Field inspection of the facility was not made.

Water Quality

Review of the water quality data submitted to JMM indicates the following:

1. A summary of coliform testing using the membrane filter method indicated that no coliform colonies were detected in 100 ml samples. Non-coliform colonies were found in less than seven percent of the samples; counts ranged from 1 to 60 colonies per 100 ml. These results do not indicate any excessive coliform contamination in the distribution system.

2. The water supplied to the study area is a 40/60 blend of State Project/Colorado River water. Chemical Quality data for the blend indicates a moderately hard water with TDS of about 500 mg/l and hardness of about 220 mg/l as $CaCO_3$.

3. Turbidity levels in the distribution system are slightly higher than the levels entering the system from the Diemer plant. Possible explanations for the difference could be:

    a. Post coagulation of alum in the transmission mains.

    b. Filter aid polymers carry through the Diemer filters.

    c. Deterioration of water quality in the water mains.

    d. Discrepencies in the measuring instruments.

Water System Operational Procedures

IRWD does not have a routine water main flushing program. Since the water is chlorinated, no problems are experienced that require flushing due to poor water quality in the distribution system. Poor water quality may result from taste and odor bearing substances, corrosive piping or stagnant conditions in dead end water mains. In addition, no sand or silt problems have been encountered that require flushing since a treated surface water is used and not well water. In areas of new construction, new water mains are chlorinated before being connected to existing lines. Coliform tests using the membrane filter method are performed on the line before approval is given to make the final line connection.

IRWD employs a full time cross-connection control inspector whose duties are to monitor all cross-connection control devices in the District, both in the industrial and irrigation areas. He keeps updated records on all cross-connection control devices and checks that they are properly maintained.

IRWD has not had problems or consumer complaints regarding taste, odors, color, rusty water, etc. No gravity or low pressure lines are found in the District's system as all old agricultural irrigation lines have been abandoned.

In terms of separation of sewer lines, reclaimed water lines, and water mains, IRWD adheres to State Health Department requirements. The SHD requires a minimum of 10 feet horizontal separation; however, in certain areas, particularly private narrow roads, only 8 feet separation is available. These variations are indicated on plans which the health department approves as part of the present district plan approval system.

Conclusions on Sanitary Evaluations of Garden Grove and IRWD Distribution Systems

Based on the information provided by both Garden Grove and IRWD, and field inspection of the Garden Grove system, it appears that no significant sanitary defects exist in the study area.

Conclusions for the City of Garden Grove study area are:

1. Well No. 6064 has minor potential of contamination due to several minor defects in well construction.
2. Water quality data indicated no violation of water quality standards, with the exception of turbidity levels in the distribution system which ranged from 0.5 to 1.0 T.U.
3. Operational procedures for the water system are adequate and in accordance with State Health Department guidelines.

Conclusions for the IRWD's system are:

1. Water served in the study is a blend of Colorado River and State Project water treated at the Diemer Treatment Plant.
2. Water quality for the study area meets all water quality standards. A slight increase was noted in the turbidity of water in the distribution system over the turbidity of water entering the system from the MWD.
3. The IRWD water system operational procedures are adequate. A slight deviation in the sewerline/water main separation guideline

occurs due to site constraints, although any deviation is received and approved by the State Health Department.

CHEMICAL AND PHYSICAL PARAMETERS

In the first sampling periods, anions and cations, trace elements, nutrients, volatile organics, and fingerprints for extractable organics were obtained. Very few peaks were observed for the extractable organics, and the source and site could not be differentiated. The IRWD source and site samples contained volatile organics, primarily trihalomethanes, which did not vary significantly with time. This was due to a relatively constant level of chlorine and TOC present in this water supply. These results are contained in Appendix A. The Garden Grove site samples contained highly variable quantities of volatile organics, primarily due to the influence of MWD water being delivered to the site. The volatile organics were not present at the well source since no chlorination of this groundwater occurred at the source and chlorine residual introduced via MWD water seldom persisted at the site. Because there appeared to be no relationship between volatile organics and the well sources, analysis for volatile organics was dropped early in the study. These analytical approaches were dropped based on observations and the initial concept of defining and predicting bacterial growth at both source and site. It should be emphasized, however, that this was a fairly crude attempt at detecting organics in drinking water. Currently, such methods as closed loop stripping and high performance liquid chromatography (HPCL) are more powerful means of examining these systems which may yield new insights on volatile organics from source to site. The idea of fingerprinting changes between sources and distribution sites deserves closer examination using newer analytical techniques.

Over the course of the study, 24 physical/chemical parameters were examined (Table 8). A summary of mean values of these physical/chemical parameters from water sampled from the two systems are summarized in Tables A1 and A2. In the first year several trends were noticed but were not confirmed over the entire study while others which were not evident in the first year surfaced when analyzing the data from the entire study. These are detailed below in the discussion of each system.

Evaluation of all the physical/chemical parameters explained nothing in relation to bacterial behavior in the distribution systems. The bulk water quality has very little predictive value for the behavior of micro-organisms in distribution systems. Perhaps a detailed look at the pipe surface did provide more useful information.

Physical parameters

The information obtained indicated that both systems had well oxygenated, rather neutral pH, and low turbidity source waters. As the water traveled from source to site the turbidity rose dramatically as did the particle counts. However, the size distribution of the particles remained very similar. The cause of the increasing concentration of particles without significant alteration of the particle size distribution is unknown at this time. The particle size distribution and concentration (particles per milliliter) for both water

TABLE 8. MAJOR PHYSICAL AND CHEMICAL PARAMETERS MEASURED

Component	Method of Analysis
Physical	
Temperature	Centigrade Thermometer
Turbidity	Meter
Redox	Meter
Particle Size Distribution	Meter
Particle Counts	Meter
Chemical	
Total Organic Carbon	Extraction
Major Cations	Flame Photometer, Titration
Major Anions	Colorimetric, Titration, Turbidimetric
General Minerals	Meter, Colorimetric
Trace Elements	Atomic Absorption

systems is shown in Table 9. The other physical parameters did not change significantly from source to site.

No significant correlations ($p < 0.05$) occurred among the six in situ variables (residual chlorine, turbidity, pH, dissolved oxygen, redox potential and temperature) in the Garden Grove system. In the IRWD system, residual chlorine correlated with pH ($r = 0.50$, $N = 84$). Dissolved oxygen and redox potential negatively correlated with temperature ($r = -0.50$, $r = -0.50$, $N = 86$, respectively). This could be expected because dissolved oxygen concentration decreases with increasing temperature, and redox potential and dissolved oxygen are intimately related.

Negative correlations were noted between the majority of physical factors and total particles and particle size distributions in the IRWD system. The opposite trend was observed in the Garden Grove system. Residual chlorine, temperature, pH, dissolved oxygen and redox potential covaried with particle size in Irvine ($r > 0.5$), while in the Garden Grove system turbidity correlated positively with particles in the > 30-40 μ and > 40-50 μ size ranges ($r = 0.5$ and 0.6, respectively, $N = 16$).

Chemical parameters IRWD

The IRWD water supply can be characterized by having a relatively high TDS (an average of 527 mg/l), high hardness (246 mg/l), moderate alkalinity (103 mg/l), high sulfate (206 mg/l) which is slightly scaline (LI = 0.20). This water has a relatively stable chlorine residual (0.73 mg/l free chlorine) with a fairly high total organic carbon (2.7 mg/l).

In order to determine which parameters required closer examination, ratios from source to site were developed for each parameter. Table 10 summarizes those parameters which have ratios < 0.75 or > 1.25. Ratios between 0.75 and 1.25 were considered within analytical error to be stable between source and site. In the first year six parameters were judged to fit this criterion (ammonia, nitrate, copper, iron, zinc, and total particles). Ammonia decreased while nitrate, copper, iron, zinc, and total particles increased from source to site. Over the entire study period there were six parameters meeting this criterion: copper, iron, manganese, silica, turbidity, and total particles. Copper decreased while silica, manganese, iron, turbidity, and total particles increased from source to site.

Copper, which MWD adds to its reservoirs for algae control, precipitated out over time. Whether the copper values observed at the source are the result of this treatment, or are corrosion products which subsequently precipitate, is not clear. Manganese, silica, and iron are probably due to corrosion products from metallic valves, other equipment and A/C pipe. The reasons for the increase in the total number of particles and turbidity from source to site are not known at this time. To some degree these values may be a reflection of increased numbers of bacteria. Increases could also be explained by inorganic particulates from corrosion and precipitation processes as water moves through the pipe. However, the relative fraction of organic to inorganic particulates cannot be determined from the existing analyses.

TABLE 9. TOTAL NUMBER OF PARTICLES PER ML AND PARTICLE SIZE DISTRIBUTION (%)

		Irvine		Garden Grove			
		Source	Site	Sources			Site
				6064	6321	23/9	
Total particles/ml		241 ± 443	1463 ± 1582	43 ± 68	30 ± 48	304 ± 358	1090 ± 1025
Channel		%	%	%			%
1	2.5 - 4 μm	36.2	30.6	38.6	38.0	38.1	28.5
2	>4 - 7 μm	41.2	37.5	39.4	37.5	39.6	37.4
3	>7 - 10 μm	10.3	11.9	9.0	9.4	9.2	12.4
4	>10 - 15 μm	7.3	10.6	7.2	7.2	7.0	11.7
5	>15 - 20 μm	2.9	4.8	3.2	3.7	2.5	4.6
6	>20 - 25 μm	1.2	2.2	1.5	1.6	1.2	2.2
7	>25 - 30 μm	0.5	1.1	0.9	0.7	0.6	1.1
8	>30 - 40 μm	0.4	0.9	0.6	0.7	0.4	1.0
9	>40 - 50 μm	0.1	0.3	0.3	0.2	0.2	0.5
10	>50 - 70 μm	0.1	0.2	0.2	0.3	0.2	0.3
11	>70 - 100 μm	0.03	0.04	0.07	0.09	0.06	0.1
12	>100 - 150 μm	0.007	0.007	0.011	0.07	0.03	0.03

TABLE 10. IRWD SOURCE TO PASEO PICASSO SITE RATIOS FOR PHYSICAL AND CHEMICAL PARAMETERS

Parameter[1]	Ratio of Mean Values Source: Site
NH_3-N	0.88
Na	1.01
K	1.00
Ca	0.96
Mg	1.07
Alkalinity	1.02
Chloride	1.02
Sulfate	1.01
Nitrate	0.84
TDS	1.01
EC[2]	1.00
Cobalt	1.10
Copper	2.43
Iron	0.22 *
Manganese	0.58 *
Silica	0.42
Zinc	1.00
Total Organic Carbon	0.88
Total particles[3]	0.16
Langelier Index	0.71

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[1] Measured in mg/l
[2] Measured in µhos/cm
[3] Measured in particles/ml

The positive Langelier Index (0.20) of this water indicates that it is slightly scaline. Therefore, calcium carbonate scale would tend to deposit on pipe surfaces and could create protected microhabitats for bacteria. The Langelier Index is a theoretical thermodynamic evaluation and does not guarantee that scale will form. Therefore, whether this is an explanation for the increased turbidity is not clear. Material from the inner surface of the pipe distribution must be examined for scale formation in order to confirm the scaling tendency predicted by the Langelier Index.

Chemical parameters - Garden Grove

Initial information from the city indicated that Wells 6321 and 6064 were the source waters for the Rockinghorse site. Comparison of the mean values for the major anions and cations from these two wells and the Rockinghorse site indicated other sources for contributing water. There was a major difference in the TDS between the wells (368 and 413 mg/l) and Rockinghorse (501 mg/l TDS). Examination of the distribution system map and subsequent conversations with the city staff indicated that Well 23/9 and possibly Well 47 contributed water to Rockinghorse. Samples were taken from both wells and Well 47 was immediately eliminated because it had a much lower TDS than 6064, 6321 and Rockinghorse. Well 23/9 had very similar TDS values and was added as one of the sampling sites in August of 1979. Further complications arose at this site because in July 1979, the city started supplementing its supply with MWD surface water to satisfy the peak summer water demands due to an unusually dry winter. During 1980, the city returned to a more normal method of operating the water system.

To reconstruct the sources for the Rockinghorse site, general mineral information from the various wells was examined and the TDS was plotted over time (Figure 5). The data indicate that during the summer months Wells 6321, 6064 and 23/9 as well as MWD water were sources for this site. Because Garden Grove does not chlorinate its supply, the occurrence of a residual at Rockinghorse implied that the site was receiving chlorinated MWD surface water. During the winter and spring months, November through May, Well 23/9 was the primary source. This was shown in the November, 1979, through May, 1980, data which closely parallel the mineral data from Well 23/9. Due to the combined sources for the Rockinghorse site, a detailed source to site analysis by the ratio approach used in the IRWD system could not be made.

Like the Irvine system, the Garden Grove system has a fairly high TDS ranging from 368-501 mg/l, high hardness (244-307 mg/l), high alkalinity (174-179 mg/l), high nitrate (13-25 mg/l) as NO_3 and is scaline with a Langelier Index of 0.37-0.4. The water pH is relatively neutral, ranging from 6.9-7.4, containing between 7-8 parts per million dissolved oxygen. The sources are relatively low in turbidity (0.2 NTU) whereas the site was relatively high having 5.0 turbidity units. The well water sources typically did not contain any chlorine residual whereas the sites did (seasonally) when MWD water was blended at the Rockinghorse site.

Table 11 summarizes the physical/chemical data for the sources and site for the Garden Grove system. Since site values were dramatically higher than the source, regardless of the blending, the conclusion is that processes in

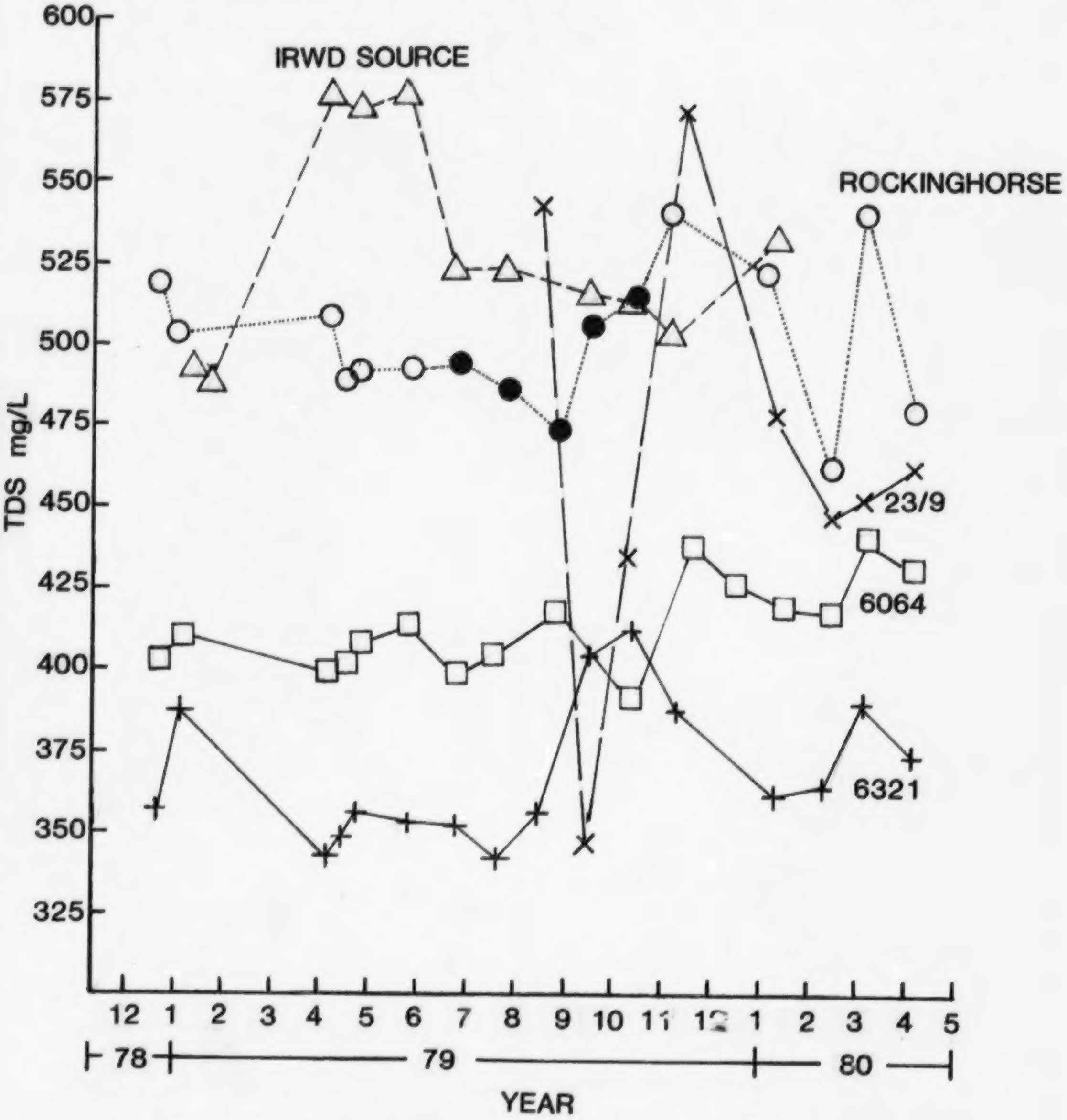

Figure 5. Description of Source Water Entering the Rockinghorse Site. Blackened circles indicate the presence of residual chlorine at Rockinghorse.

TABLE 11. MEAN PHYSICAL/CHEMICAL VALUES FOR GROUNDWATER

	Sources			
Parameter (mg/l)	6321	6064	23/9	Site
TDS	368	413	468	500
NH_3-N	0.023	0.023	0.038	0.05
Residual Chlorine	0	0	0	0
Fe	0.016	0.013	0.042	0.160
TOC	0.53	0.50	0.50	1.0
D.O.	7.4	7.7	7.9	7.1
pH (units)	7.4	7.2	6.9	7.4
Turbidity (NTU)	0.23	0.27	0.30	5.0
Total Particles (#/ml 2.5-150 μ)	44	30	304	1090

the distribution system must have been responsible for the values observed at the site. These parameters are iron, TOC, turbidity and total particles (TOC is probably not significantly different but due to imported MWD water). Similar to the IRWD system, increases in iron, turbidity and total particles probably are due to the same processes as described in the previous section.

BACTERIOLOGICAL EVALUATION

Plating Techniques and Bacterial Enumerations

Pour plates have classically been employed for quantitative microbiology. This method is slowly being replaced with the spread plate or membrane filter technique for several reasons. For example, data from this investigation (Table 12) indicated that the pour plate technique was the least efficient in recovery of total bacteria. Importantly, beyond the temporal and monetary considerations lies the effectiveness of the method to recover bacteria from water.

Related to recovery efficacy is incubation temperature, length of incubation and the bacterial population of interest. The media and methods shown in Table 5 were examined with the above considerations in mind. Mueller-Hinton and trypticase Soy Agar were discontinued early in the study due to their relatively poor recoveries of bacteria. The 20°C incubation temperatures were included in the study to determine the importance of pigmented bacteria in water distribution systems.

The data in Table 12 show the ability of the different plating techniques and media to recover bacteria from the two distribution systems. As expected, 20°C incubation temperatures were more favorable for the recovery of pigmented bacteria from these water distribution systems. The *Standard Methods* approved standard total plate count using pour plates nearly always produced pigmented counts that were at least 50% less than the counts obtained using the spread plate technique. This could be attributed to the stressing of sensitive bacteria by the elevated temperature of the agar, preventing colony formation. Also, many of the bacterial isolates might be strongly aerophilic and experience reduced or no growth under low oxygen tension conditions within the agar. Lastly, pigmented colonies are inherently difficult to identify when embedded in agar. The failure of the pour plate method to corroborate higher counts obtained on the other media was repeatedly encountered during the course of this study.

It is also clear from Table 12 that regardless of the medium used, an increase in total and pigmented bacterial counts occurred between the source and site in each system. These data suggest that regrowth does occur even in a highly chlorinated system such as Irvine. Alternatively, this increase between source and site in Irvine may be due to cells regaining viability as they move from areas of high chlorine residuals to relatively low chlorine residual areas. As would be expected in most instances the total bacterial counts obtained were higher in the unchlorinated Garden Grove System.

The highest counts were obtained using MSPC medium in both systems, generally followed by R-2A low nutrient medium and TPC35SP. In 98 of 123

TABLE 12. MEAN BACTERIAL CONCENTRATIONS (#/ml) AT
WATER DISTRIBUTION SOURCE AND SITE SAMPLING LOCATIONS

Plate Count Technique	Irvine				Garden Grove			
	Source	(N)	Site	(N)	Sources	(N)	Site	(N)
TPC35SP	70.0	(18)	327.3	(148)	159.7	(40)	740.2	(137)
TPCP35SP	7.6	(18)	30.7	(121)	61.1	(40)	222.2	(118)
TPC35PP	38.4	(16)	107.4	(106)	119.9	(41)	500.7	(105)
TPCP35PP	0.4	(17)	17.0	(104)	21.5	(41)	29.2	(100)
TPC20PP	44.6	(12)	123.5	(82)	124.7	(23)	513.9	(78)
TPCP20PP	11.0	(12)	17.0	(81)	13.7	(23)	80.1	(77)
TPC20SP	65.6	(17)	192.8	(95)	120.7	(40)	765.1	(92)
TPCP20SP	14.0	(16)	48.5	(95)	36.4	(40)	355.9	(92)
LOWNUT	69.2	(16)	352.8	(94)	260.7	(40)	1200.4	(91)
LOWNUTP	21.3	(16)	69.4	(94)	108.7	(40)	366.9	(90)
m-SPCT	273.4	(15)	721.8	(82)	467.8	(39)	1810.4	(84)
m-SPCP	194.7	(15)	415.5	(78)	236.4	(38)	833.4	(79)

TPC35SP = Total Plate Count Medium 35°C spread plate: Total Count
TPCP35SP = Total Plate Count Medium 35°C spread plate: pigmented
TPC35PP = Total Plate Count Medium 35°C pour plate: Total Count
TPCP35PP = Total Plate Count Medium 35°C pour plate: pigmented
TPC20PP = Total Plate Count Medium 20°C pour plate: Total Count
TPCP20PP = Total Plate Count Medium 20°C pour plate: pigmented
TPC20SP = Total Plate Count Medium 20°C spread plate: Total Count
TPCP20SP = Total Plate Count Medium 20°C spread plate: pigmented
LOWNUT = Low Nutrient
LOWNUTP = Low Nutrient: pigmented
m-SPCT = M- Standard Plate Count Medium: Total Count
m-SPCP = M- Standard Plate Count Medium: Pigmented Count

matched comparisons for both Irvine and Garden Grove, R-2A medium produced total counts 2 to 5 times greater than TPC35SP.

Lower counts were obtained using 20°C incubated spread plates on total plate count medium and could have been due to the slower growth of bacteria at this incubation temperature. The poorest recoveries were noted using the pour plate technique. The fact that both m-SPC and R-2A media have longer incubation periods suggests that many of the bacteria require longer than 48 hours to produce visible colonies. The TPC35SP methodology limits the incubation time for slow-growers; consequently, counts were only about 50% of the counts obtained with 96 hour incubation on m-SPC medium.

An important question addressed later in the text relates to the nature of the bacterial population isolated as a result of medium composition and incubation temperature. Investigation of this aspect seems particularly important in light of the results presented in Table 12.

Bacterial recovery for all the media was compared to m-SPC medium results (Table 13). The ratios in this table are based on arithmetic means for each of the test media. R-2A recovery efficiency (.87) compared well to m-SPC for total counts in the Garden Grove system, but was only slightly better (.41) than plate count agar spread plates (.34) incubated at 35°C for the recovery of the pigmented population. The efficiency of the media for recovering total and pigmented bacteria varied between the two systems. In the chlorinated system the efficiency of recovery decreased for all media tested. Importantly, the Standard Methods pour plate technique (118) underestimated the total bacterial population (as estimated by the m-SPC count) by 79 percent in the chlorinated system and by 66 percent in the unchlorinated Garden Grove system. The pigmented bacterial population was even further underestimated, pour plate counts being 88 and 97 percent lower than m-SPC counts in the chlorinated and non-chlorinated systems, respectively (Table 13).

M-SPC medium incubated at 35°C recovered greater numbers of pigmented bacteria than did R-2A at 20°C (Figure 6). The recovery of pigmented bacteria on R-2A and TPC20SP was similar even though R-2A medium was incubated 24 hours longer than TPC agar spread plates incubated at 20°C. The extended incubation period of R-2A medium appeared to be of limited usefulness in light of the small post-48 hour increase in recovery.

When enumeration of pigmented bacteria is a desirable measure of water quality, it is clear that the isolation medium must be chosen with concern for optimal recovery. For this reason it is suggested that a standard medium be designated for the quantification of pigmented bacteria in water.

Analysis of the Irvine System data by stepwise multiple regression indicated that of the six physical and chemical parameters (residual chlorine, pH, EC, turbidity, temperature and EH) only pH was a significant contributing factor in fluctuations of both pigmented and total counts only on R-2A low nutrient medium and accounted for 8.8 and 9.5% of the variation respectively. The reason for this in unclear. In the Garden Grove System redox, pH, water temperature and electroconductivity significantly influenced total counts on TPC35PP and pH pigmented counts on TPC20SP.

TABLE 13. RATIO OF BACTERIAL RECOVERY OF MEDIA STANDARDIZED TO M-SPC MEDIUM

	m-Standard Plate Count	Low Nutrient R-2A	Plate Count Agar 35°C Spread Plates	Plate Count Agar 20°C Spread Plates	Plate Count Agar 20°C Pour Plates	Plate Count Agar 35°C Pour Plates
TOTAL						
GG Sites	1.00	.87	.46	.47	.42	.34
Systems Combined	1.00	.72	.40	.37	.32	.27
IRWD Sites	1.00	.61	.37	.30	.25	.21
PIGMENTED						
GG sites	1.00	.41	.34	.31	.21	.13
Systems Combined	1.00	.32	.26	.27	.14	.13
IRWD Sites	1.00	.27	.18	.21	.10	.12

An analysis of the TPCP35SP counts was made to determine site variability in the pigmented bacterial population within the system. The data indicated that there were marked differences between the sites at Garden Grove, but not Irvine. A one-way analysis of variance of the various media by the different sites indicated that no difference existed between the Irvine sites for TPC35SP, TPC35P, TPCP35PP, TPCP20SP, TPCP20PP, LNT, LNP, MSPCP and MSPCT. Differences were found between total counts on TPC35PP at Paseo Picasso and Via Messina ($T = 2.5$, d.f. $= 12$ $\alpha < 0.05$). These data point to the uniformity of the bacterial population at Irvine relative to the Garden Grove system. The sources were excluded from this discussion due to the small number of samples collected.

Further T-test results (Table 14) between mean bacterial counts (Figure 6) revealed that the m-SPC filtration method with a 96 hour incubation recovered significantly higher total and pigmented bacterial numbers than other media and techniques tested. In Garden Grove (Table 14) R-2A medium recovered statistically similar numbers of bacteria when compared to m-SPC medium, while in the IRWD system (Table 14) m-SPC medium recovered significantly more bacteria than did R-2A medium.

Plate count agar (PC, Difco) utilizing the spread plate technique incubated at both 20°C and 35°C produced statistically similar geometric means for pigmented and total counts in Garden Grove (Figure 6). However, in the IRWD system, incubation temperature significantly affected the total and pigmented counts were lower in the chlorinated system, but proportionately more pigmented bacteria were recovered when compared to the unchlorinated system (Figure 6). The systems differ in the optimum incubation temperature required for maximum bacterial recovery. One explanation for these findings could be that chlorination practices in the IRWD system may increase surviving cells' sensitivities to different incubation temperatures.

Chlorine appeared to affect the composition of the bacterial population in the water distribution system. Data on the prevalence of pigmented bacteria related to free chlorine residuals is shown in Table 15. The ratio of pigmented to total bacteria reflected the relative ability of the respective media to recover this segment of the bacterial population at each free residual chlorine range. When free chlorine residuals of $>0.55 \le 0.7$ mg/l were present, the observed proportion of pigmented bacteria in the population increased (due to a decrease in the total bacterial count) regardless of the medium used. At free chlorine levels >0.7 mg/l the proportion of pigmented bacteria decreased, but in most instances was still more than double that observed at low chlorine levels. If bacteria possess differential susceptibility to disinfection by free chlorine, either due to differences in cell wall components or growth and attachment characteristics, susceptible subcomponents of the bacterial population would be expected to fluctuate in relation to chlorine levels. These data suggested that chlorine may influence the survivability of various bacterial strains and/or the ability of non-selective media to recover chlorine-shocked bacteria.

In this study the media recovered significantly different numbers of bacteria from the same water samples. This implies that what are considered non-selective media are, indeed, quite selective. Each of these media contain

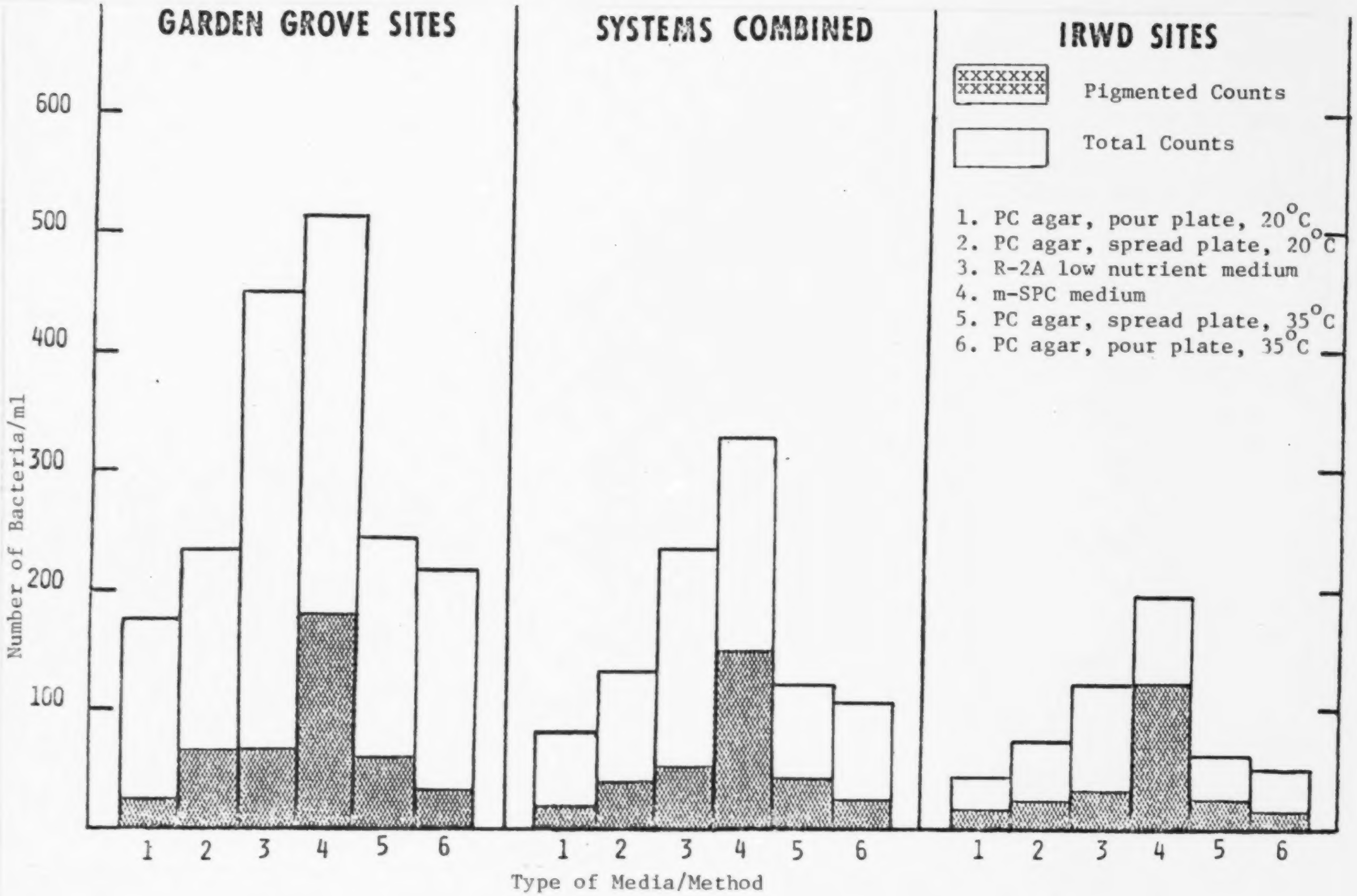

Figure 6. Geometric Means of Total and Pigmented Bacterial Counts from Garden Grove and Irvine, California

TABLE 14. T-TEST ANALYSIS AMONG ARITHMETIC PIGMENTED MEAN BACTERIAL COUNTS RECOVERED ON TEST MEDIA

	Garden Grove					
	m-SPC	LN R-2A	PC35P	PC20SP	PC35PP	PC20PP
m-SPC	–	NS	>	>	>	>
LNR-2A	<	–	>	>	>	>
PC35SP	<	NS	–	NS	>	>
PC20SP	<	NS	NS	–	>	NS
PC35PP	<	<	<	<	–	NS
PC20PP	<	<	<	<	<	–
	Irvine					
m-SPC	–	>	>	>	>	>
LNR-2A	<	–	NS	>	>	>
PC35SP	<	<	–	>	>	>
PC20SP	<	NS	>	–	NS	NS
PC35PP	<	<	<	<	–	NS
PC20PP	<	<	<	<	NS	–

NS = Not significant at .05 level of probability
< = Significantly less at .05 level of probability
> = Significantly greater at .05 level of probability

TABLE 15. REDISUAL CHLORINE LEVELS AND PIGMENTED/TOTAL BACTERIA RATIOS FOR IRVINE AND GARDEN GROVE COMBINED SYSTEMS

	Free Residual Chlorine (mg/l)		
Medium	>0.1 < 0.55	>0.55 < 0.7	>0.7
m-SPC	.26	.72	.57
R-2A	.09	.30	.22
PC35SP	.17	.35	.23
PC35PP	.06	.24	.20
PC20SP	.11	.27	.39
PC20PP	.06	.17	.12

particular growth requirements and m-SPC (Table 6), by far the simplest of the three media, recovered the highest numbers of total and pigmented bacteria. M-SPC also contains relatively large amounts of the basic growth requirements of heterotrophic bacteria. It affords cells approximately four times more (by weight) amino acids than Plate Count agar and twenty times more than R-2A. Gram for gram, considerably more carbon source is included in m-SPC (10.0 ml glycerol) than in Plate Count agar (1.0 g glucose) and R-2A (0.5 g glucose, 0.5 g soluble starch and 0.5 g sodium pyruvate). R-2A agar supplies trace quantities of a number of supplemental nutrients (primarily to enhance pigment production by bacteria) in addition to two amino acid and three carbohydrate sources. It is likely that R-2A medium recovers more bacterial genera than the other media because of the wide variety of nutrients it supplies. The m-SPC medium may be more effective in the recovery of injured bacteria in the water sample, because it provides increased amounts of rich nutrient sources and, as a result, develops higher colony counts. Alternatively, the presence of these large amounts of basic nutrients may encourage the rapid growth of less fastidious bacteria, thus giving greater counts but a lower diversity of bacterial species than are actually present.

Spearman Rank Correlation Coefficients on Irvine and Garden Grove Data

The Spearman Rank Correlation Coefficient (SRCC or r_s) is a nonparametric measure of association between two variables. The SRCC is obtained by ranking the individual values of the variable from highest (1) to lowest (n), subtracting and squaring the differences between paired ranks of the two variables (d) and summing the squares. The overall equation for the SRCC is as follows:

$$r_s = 1 - \frac{6\overset{n}{\underset{i = 1}{}} \; d_i^{2}}{n(n^2 - 1)}$$

Values of the SRCC range from +1 to -1. An examination of the results of a computer analysis generating r_s for all pairings of media employed in this study revealed definite differences between the Irvine and the Garden Grove systems. In Tables 16, 17, and 18 the frequencies of significant SRCC's that occurred among the media for each system individually and the two systems combined are shown. A total of 68 significant SRCC's ($r_s > 0.5$) were obtained between media for Garden Grove samples while only 46 were obtained for the Irvine samples. The maximum possible number of correlations is given by the binomial coefficient, $\overset{n}{k} = \frac{n \qquad x}{k \;\; 9n - k}$ (2), where n = number of media, k = size of the sample, and the constant "(2)" is included to take into consideration reciprocal relationships. This value is 132 for both systems. A McNemar test comparing the two SRCC frequencies for both systems produced a Chi-square value statistically significant at ($p = 0.014$). In Table 19 a 2X2 contingency table representing frequencies of significant and non-significant SRCC's between media in the two systems is shown. The media ranked similarly in both systems in 69.7% of the comparisons (27/66). That is, a non-significant (or significant) SRCC in Garden Grove was followed by a similar result in Irvine. Of the remaining non-similar rankings (30.3%), 24.2% involved instances where significant SRCC's were found in Garden Grove and non-significant ones in Irvine. The relatively large number of significant SRCC's at Garden Grove

TABLE 16. FREQUENCIES OF SPEARMAN RANK CORRELAION COEFFICIENTS AMONG MEDIA IN GARDEN GROVE

Method	Frequency of Correlations ($r_s > .5$)	Combined Pigmented/ Total
TPC35SP	8	16
TPCP35SP	8	
TPC35PP	7	
TPCP35PP	4	11
TPC20SP	7	
TPCP20SP	8	15
mSPCT	4	
mSPCP	1	5
TPC20PP	7	
TPCP20PP	2	9
R-2AT	7	
R-2AP	5	12
	Total	68*

*Total Spearman Rank Correlations between all media counts at Garden Grove.

TPC35SP	Total Plate Count Medium 35°C Spread Plate:	Total Count
TPCP35SP	Total Plate Count Medium 35°C Spread Plate:	Pigmented
TPC35PP	Total Plate Count Medium 35°C Pour Plate:	Total Count
TPCP35PP	Total Plate Count Medium 35°C Pour Plate:	Pigmented
TPC20SP	Total Plate Count Medium 20°C Spread Plate:	Total Count
TPCP20SP	Total Plate Count Medium 20°C Spread Plate:	Pigmented
TPC20PP	Total Plate Count Medium 20°C Pour Plate:	Total Count
TPCP20PP	Total Plate Count Medium 20°C Pour Plate:	Pigmented
mSPCT	m-Standard Plate Count Medium:	Total Count
mSPCP	m-Standard Plate Count Medium:	Pigmented
R-2AT	Low Nutrient R-2A Medium:	Total Count
R-2AP	Low Nutrient R-2A Medium:	Pigmented

TABLE 17. FREQUENCIES OF SPEARMAN RANK CORRELATIONS AMONG MEDIA IN IRVINE

Method	Frequency of Correlations ($r_s > .5$)	Combined Pigmented/ Total
TPC35SP	6	
TPCP35SP	2	8
TPC35PP	6	
TPCP35PP	3	9
TPC20SP	6	
TPCP20SP	5	11
mSPCT	1	
mSPCP	1	2
TPC20PP	6	
TPCP20PP	2	8
R-2AT	4	
R-2AP	1	5
	Total	43*

*Total Spearman Rank Correlations between all media counts at Irvine.

TPC35SP	Total Plate Count Medium 35°C Spread Plate:	Total Count
TPCP35SP	Total Plate Count Medium 35°C Spread Plate:	Pigmented
TPC35PP	Total Plate Count Medium 35°C Pour Plate:	Total Count
TPCP35PP	Total Plate Count Medium 35°C Pour Plate:	Pigmented
TPC20SP	Total Plate Count Medium 20°C Spread Plate:	Total Count
TPCP20SP	Total Plate Count Medium 20°C Spread Plate:	Pigmented
TPC20PP	Total Plate Count Medium 20°C Pour Plate:	Total Count
TPCP20PP	Total Plate Count Medium 20°C Pour Plate:	Pigmented
mSPCT	m-Standard Plate Count Medium:	Total Count
mSPCP	m-Standard Plate Count Medium:	Pigmented
R-2AT	Low Nutrient R-2A Medium:	Total Count
R-2AP	Low Nutrient R-2A Medium:	Pigmented

TABLE 18. FREQUENCIES OF SPEARMAN RANK CORRELATION COEFFICIENTS AMONG MEDIA IN IRVINE AND GARDEN GROVE SYSTEMS COMBINED

Method	Frequency of Correlations ($r_s > .5$)	Combined Pigmented/ Total
TPC35SP	6	
TPCP35SP	2	10
TPC35PP	6	
TPCP35PP	3	8
TPC20SP	6	
TPCP20SP	5	13
mSPCT	1	
mSPCP	1	5
TPC20PP	6	
TPCP20PP	2	11
R-2AT	4	
R-2AP	1	8
	Total	57*

*Total Spearman Rank Correlations between media in both systems combined.

TPC35SP	Total Plate Count Medium 35°C Spread Plate:	Total Count
TPCP35SP	Total Plate Count Medium 35°C Spread Plate:	Pigmented
TPC35PP	Total Plate Count Medium 35°C Pour Plate:	Total Count
TPCP35PP	Total Plate Count Medium 35°C Pour Plate:	Pigmented
TPC20SP	Total Plate Count Medium 20°C Spread Plate:	Total Count
TPCP20SP	Total Plate Count Medium 20°C Spread Plate:	Pigmented
TPC20PP	Total Plate Count Medium 20°C Pour Plate:	Total Count
TPCP20PP	Total Plate Count Medium 20°C Pour Plate:	Pigmented
mSPCT	m-Standard Plate Count Medium:	Total Count
mSPCP	m-Standard Plate Count Medium:	Pigmented
R-2AT	Low Nutrient R-2A Medium:	Total Count
R-2AP	Low Nutrient R-2A Medium:	Pigmented

TABLE 19. FREQUENCIES OF SIGNIFICANT AND NON-SIGNIFICANT SRCC'S BETWEEN MEDIA IN THE TWO WATER DISTRIBUTION SYSTEMS

		IRVINE	
		Significant	Non-Significant
GARDEN GROVE	Significant	19	16
	Non-Significant	4	27

n = 66
Chi-square = 6.05
p = .014

suggests that the media maintain an hierarchical order of recovery efficiencies in this system. The major contributors to the difference between Irvine and Garden Grove SRCC frequencies are R-2A and R-2AP, and TPC35SP total and pigmented counts. In Garden Grove, 12 SRCC's were obtained for the R-2A and R-2AP counts as compared to 5 for the same medium at Irvine. Sixteen TPC35SP and TPCP35SP SRCC's were recorded at Garden Grove as compared to 8 for the same two media at Irvine.

The spread plate technique provided more consistant results in the Garden Grove system, that is, it most closely followed the expected hierarchy of recovery efficiencies. The pour plate technique appeared to rank similarly in both systems (Tables 16 and 17).

Results obtained earlier in this study indicated that the various media had different recovery efficiencies. These efficiencies could generally be arranged in a particular order based on the plate count results. Seemingly random fluctuations in the hierarchical ranking of media recovery efficiencies may, in fact, be far from random. Instead, some parameter operating in the system may account for these observations. The use of chlorine disinfection at Irvine may explain this variance. Transient fluctuations in the viability of the bacterial population at Irvine occurred as a result of chlorination. It is conceivable that individual cells may incur damage to very different physiological functions. Repair of the damage required specific nutrients. The availability of these nutrients in the culture medium is critical to good recovery efficiency of the medium. In addition, all bacterial genera in the system have optimum growth temperatures. The recovery of injured organisms may be facilitated by specific incubation temperatures. As a result, one medium (or method) is favorable to the repair of a particular cell component or function while another offers no recovery advantage. The issue is further confounded by individual species requirements, susceptibilities to chlorine and fluctuations in the species composition of the bacterial population present in the water over time.

The pour plate and spread plate methods using identical media often produce significantly different results. Apparently some aspect of plate pouring decreases bacterial numbers; this decrease is not seen or is much less in using the spread plate method. There are two major anomalies in the pour plate method; the first involves immersion of bacteria in the water samples in warm agar. It is well documented that heat stress can seriously impair bacterial growth and extend the normal lag phase. In addition, minimal media which are nutritionally adequate for the growth of physiologically healthy bacteria may not support the growth of heat-stressed bacteria. This may especially be the case when bacteria have a low optimum temperature, e.g., certain *Pseudomonas* are abundant in the systems being studied. Unfortunately, the temperature differential between the two systems does not vary enough to directly test the hypothesis: that pour plate recovery of bacteria is inversely related to the magnitude of the psychrophilic sub-population in the water system. The second anomalie is the oxygen demand of pour plate agar. Obligate aerobes may be suppressed by immersion in agar, but the degree to which this occurs in practice is unknown.

Relatively constant bacterial recoveries on a medium reflect a bacteriologically-stable water distribution system. In light of this, Garden Grove appeared to have a bacteriologically-stable water supply compared to the IRWD system. This difference may be due, in part, to chlorination practices in the Irvine system. Garden Grove did not have a routine chlorination program, unlike the IRWD system, where free chlorine residuals were commonly 0.5 mg/l and occasionally recorded levels of 1.0 mg/l. Ridgway and Olson (106) have shown that variations in combined residual chlorine may influence the generic make-up of viable bacteria in these systems and, as a result, could cause discrepancies in the recovery efficiencies of nutritionally different media. These discrepancies can be detected by using the SRCC analysis.

Ecological succession patterns in unperturbed systems generally range from "unstable" to "stable". Frequently, chronological age dictates the stage of succession encountered and this, in turn, varies with the nature of the climax state and the initial quality of the habitat (105). The Irvine system was installed in 1962, while the Garden Grove system was in use in the early 1920's. If bacteriological succession patterns indeed occur in water distribution systems and barring any perturbations such as pipe replacement, the expected result would be that the Garden Grove system is microbiologically closer to a stable climax community than the IRWD system. The SRCC values support this hypothesis.

Pearson's r Correlation Coefficients (Tables 20 and 21) were computed between the bacterial counts on the different media in both systems. Of foremost interest was the apparent lack of correlations for m-SPC medium with any of the other media tested in the IRWD system. In Garden Grove, m-SPC counts correlated well with counts obtained on PC agar spread plates incubated at 35°C and 20°C but not with either pour plate method or R-2A medium. The nutrient content of the media listed in Table 6 indicated m-SPC and PC agar are more similar in composition than either are to R-2A medium. The lack of correlations between m-SPC and R-2A, and between R-2A and Plate Count agar suggested that different media were selecting different bacterial genera within the total bacterial population, or a difference in the degree of recovery of stressed or injured organisms had occurred. Those genera with very specific nutritional requirements would grow better on a medium such as R-2A that supplies a wide variety of biochemicals.

PC agar pour plate counts correlated well with spread plate counts indicating media composition is more important than plating method in obtaining statistically consistent results in these systems. The high degree of correlation between 20°C and 35°C pour plates in both systems suggests that this technique was consistent, but the selective effects of the pour plate method (heat stress and oxygen tension) produced low total and pigmented bacterial counts and therefore reduced its value for accurately reflecting bacterial counts in these two systems.

One way analysis of variance performed on the data from the Garden Grove samples indicated significant differences between the Harris site and the other test sites (Table 22). Harris had consistantly higher counts than the samples from the other sites. No differences between sites were observed on the basis of TPC35PP, TPC20SP, R-2A, MSPCT and MSPCP counts. Interestingly,

TABLE 20. PEARSON'S r CORRELATION COEFFICIENTS BETWEEN PLATE COUNT MEDIA AND PLATING TECHNIQUES FOR IRVINE RANCH WATER DISTRIBUTION SYSTEM

	PC35SP	PCP35SP	PC20SP	PCP20SP	PC35PP	PCP35PP	PC20PP	PCP20PP	m-SPCT	m-SPCP	R-2A	R-2AP
PC35SP	1.0	.88 (122)	.82 (95)	.78 (95)	.50 (106)	NS	.67 (96)	NS	.69 (87)	NS	NS	.51 (95)
PCP35SP		1.0	.57 (50)	.64 (95)	NS (103)	.48 (96)	.52	NS	.48 (87)	NS	NS	NS
PC20SP			1.0	.78 (97)	.66 (95)	NS	.76 (96)	.53 (95)	.63 (95)	NS (79)	.54 (96)	.62 (95)
PCP20SP				1.0	NS	NS	.70 (96)	NS	.65 (79)	.56 (77)	NS	.57 (95)
PC35PP					1.0	NS	.77 (96)	.91	NS	NS	.62	.77
PCP35PP						1.0	.49 (96)	NS	NS	NS	NS	NS
PC20PP							1.0	.57 (97)	.50 (80)	NS	NS	NS
PCP20PP								1.0	NS	NS	.58 (96)	.77 (95)
m-SPCT									1.0	NS	NS	.68 (97)
m-SPCP										1.0	NS	NS
R-2A											1.0	NS
R-2AP												1.0

PC35SP = Plate Count Medium 35°C Spread Plate: Total Count
PCP35SP = Plate Count Medium 35°C Spread Plate: Pigmented
PC20SP = Plate Count Medium 20°C Spread Plate: Total Count
PCP20SP = Plate Count Medium 20°C Spread Plate: Pigmented
PC35PP = Plate Count Medium 35°C Pour Plate: Total Count
PCP35PP = Plate Count Medium 35°C Pour Plate: Pigmented
PC20PP = Plate Count Medium 20°C Pour Plate: Total Count

(continued)

TABLE 20 (continued)

PCP20PP = Plate Count Medium 20°C Pour Plate: Pigmented
R-2A = Low Nutrient R-2A Medium 20°C Spread Plate: Total Count
R-2AP = Low Nutrient R-2A Medium 20°C Spread Plate: Pigmented
m-SPCT = m-Standard Plate Count Medium 35°C Membrane Filtration: Total Count
m-SPCP = m-Standard Plate Count Medium 35°C Membrane Filtration: Pigmented
( ) = n
NS = Not Significant at the .001 Level of Probability

TABLE 21. PEARSON'S r CORRELATION COEFFICIENTS BETWEEN PLATE COUNT MEDIA AND PLATING TECHNIQUES FOR GARDEN GROVE WATER DISTRIBUTION SYSTEM

	PC35SP	PCP35SP	PC20SP	PCP20SP	PC35PP	PCP35PP	PC20PP	PCP20PP	m-SPCT	m-SPCP	R-2A	R-2AP
PC35SP	1.0	.40 (118)	.64 (94)	NS	.48 (102)	NS	.51 (92)	NS	NS	NS	NS	NS
PCP35SP		1.0	NS	NS	NS	NS	NS	.41 (91)	NS	NS	NS	NS
PC20SP			1.0	.51 (93)	.64 (91)	NS	.71 (92)	NS	NS	NS	.71 (92)	NS
PCP20SP				1.0	NS	NS	.51 (91)	.47 (90)	NS	NS	NS	NS
PC35PP					1.0	.62 (100)	.92 (90)	NS	NS	NS	NS	NS
PCP35PP						1.0	.55 (90)	NS	NS	NS	NS	NS
PC20PP							1.0	.50 (91)	NS	NS	.52 (90)	NS
PCP20PP								1.0	NS	NS	NS	NS
m-SPCT									1.0	.87 (74)	NS	NS
m-SPCP										1.0	NS	NS
R-2A											1.0	.57 (92)
R-2AP												1.0

PC35SP = Plate Count Medium 35°C Spread Plate: Total Count
PCP35SP = Plate Count Medium 35°C Spread Plate: Pigmented
PC20SP = Plate Count Medium 20°C Spread Plate: Total Count
PCP20SP = Plate Count Medium 20°C Spread Plate: Pigmented
PC35PP = Plate Count Medium 35°C Pour Plate: Total Count
PCP35PP = Plate Count Medium 35°C Pour Plate: Pigmented
PC20PP = Plate Count Medium 20°C Pour Plate: Total Count

(continued)

TABLE 21 (continued)

PCP20PP = Plate Count Medium 20°C Pour Plate: Pigmented
R-2A = Low Nutrient R-2A Medium 20°C Spread Plate: Total Count
R-2AP = Low Nutrient R-2A Medium 20°C Spread Plate: Pigmented
m-SPCT = m-Standard Plate Count Medium 35°C Membrane Filtration: Total Count
m-SPCP = m-Standard Plate Count Medium 35°C Membrane Filtration: Pigmented
( ) = n
NS = Not Significant at the .001 Level of Probability

TABLE 22. ONE WAY ANALYSIS OF VARIANCE OF PLATE COUNT MEDIA AND SITES IN THE GARDEN GROVE WATER DISTRIBUTION SYSTEM

Medium	Sites	T	α
Total Plate Count Medium* 35°C Spread Plate	Sandra/Harris	-3.2	≤0.05
Total Plate Count Medium Pigmented 35° Spread Plate	Sandra/Harris	-2.2	≤0.05
Total Plate Count Medium Pigmented 35°C Pour Plate	Sandra/Harris	-2.2	≤0.05
Total Plate Count Medium Pigmented 20°C Spread Plate	Sandra/Harris	-2.9	≤0.05
Total Plate Count Medium Pigmented 20°C Spread Plate	Rockinghorse/Harris	-2.6	≤0.01
Total Plate Count Medium* 20°C Pour Plate	Sandra/Harris	-3.0	≤0.05
Total Plate Count Medium* 20°C Pour Plate	Rockinghorse/Harris	-2.4	≤0.05
Total Plate Count Medium Pigmented 20°C Pour Plate	Sandra/Harris	-2.4	≤0.05
Total Plate Count Medium Pigmented 20°C Pour Plate	Rockinghorse/Harris	-2.0	≤0.01

α = Level of Significance
d.f. = degrees of freedom (separate variance)

there were similarities between three of the four sites, but the Harris site was markedly different. The key feature of this difference at the Harris site was attributable to high total and pigmented counts (Table 24). These data (Table 24) clearly indicate that m-SPC medium is more variable between sites; thus less ability to show any statistical difference regarding counts between the sites would be expected.

Bacterial Enumeration in Irvine and Garden Grove

Bacterial counts obtained using the six plating techniques were compared for each sampling location using geometric mean counts from July 1978 through April 1980 (Tables 23, 24). The date of initiation of each plating method can be found in Table 5. The data in Table 23 indicate that in the IRWD samples, the highest mean total bacterial counts were obtained at L. Parkview Lane for all media excluding m-SPC. At Paseo Picasso the media ranked in order of highest counts yielded were, m-SPC, TPC35SP, and low nutrient R-2A. At Via Messina total counts were approximately equal for all media except TPC20SP, which yielded counts approximately 50% lower than the other media. The lower incubation temperature and the shorter 48 hour incubation time may explain this finding. At L. Parkview Lane TPC35SP gave the highest total counts followed in order of decreasing mean value by m-SPC, R-2A, TPC20SP, TPC20PP, and TPC35PP. Arbor Lane yielded the highest counts on m-SPC followed by low nutrient R-2A medium and TPC35SP. TPC20SP and TPC20PP were approximately equal in bacterial recovery and accounted for 1/5 and 1/2 of the total mean bacterial values obtained on m-SPC and R-2A medium, respectively.

In the Garden Grove System m-SPC was the best medium for enumerating total and pigmented bacteria at all sampling locations (Table 24). Harris Street showed the highest overall mean total and pigmented bacterial counts across all media tested. Rockinghorse and Marshall Lane produced similar bacterial numbers. Sandra No. 2 had lower counts than the other sites except on M-SPC and reflected the smallest increase from source to site. Pigmented bacterial counts at Sandra No. 2 and Rockinghorse decreased or remained constant in most instances from source to site. The m-SPC medium recovered similar bacterial numbers from all the various sites. This contrasts greatly with results obtained for other media, i.e., TPC35SP. The m-SPC medium was also superior in the recovery of pigmented bacteria in the Garden Grove system. The increase in bacterial numbers from source to site is shown in the data in Table 25.

The data in Table 26 and that in Appendix A (Tables A3-7) indicate that high bacterial numbers can exist in the presence of high free residual chlorine, and raise the question of how these organisms survive in such systems. Different locations can appear to differ or be similar in bacterial counts depending on a given medium. At Garden Grove m-SPC medium illustrates this point. If only m-SPCmedium was used to evaluate bacterial counts the sites would be judged to be similar, while if TPC35SP data was used, the sites would appear to be very different. Irvine data (Table 23) did not give uniform counts on m-SPC medium across sites, but when ranked by the order of bacterial counts and by medium at the different sites, the data reflect the same problem as indicated by the Garden Grove data. These results suggest that different bacterial genera are selected by different media and raise the question of

TABLE 23 MEAN BACTERIAL CONCENTRATIONS (#/ml) IN THE
IRVINE RANCH WATER DISTRIBUTION SOURCE & SITE LOCATIONS

Plate Count Technique	Source	(N)	Sites							
			Paseo Picasso	(N)	Via Messina	(N)	L.Parkview	(N)	Arbor	(N)
TPC35SP*	70.0	(18)	61.7	(42)	84.1	(9)	102.2	(39)	72.6	(30)
TPCP35SP	7.6	(18)	17.7	(36)	24.5	(25)	25.8	(33)	33.1	(24)
TPC35PP*	38.4	(16)	33.6	(32)	75.6	(23)	46.2	(30)	51.6	(21)
TPCP35PP	0.4	(17)	11.4	(32)	19.2	(22)	16.4	(30)	15.6	(20)
TPC20SP*	65.6	(17)	63.2	(29)	79.2	(19)	60.2	(26)	84.5	(20)
TPCP20SP	14.0	(16)	28.7	(29)	17.2	(19)	43.5	(26)	44.1	(20)
TPC20PP*	44.6	(12)	24.9	(21)	93.0	(20)	51.6	(21)	79.4	(20)
TPCP20PP	11.0	(12)	9.7	(20)	13.7	(20)	14.8	(21)	16.5	(20)
R-2A*	69.2	(16)	138.2	(29)	165.7	(20)	121.2	(26)	225.1	(20)
R-2A Pigmented	21.3	(16)	34.8	(29)	39.5	(20)	29.7	(26)	59.1	(20)
m-SPC*	273.4	(15)	219.4	(25)	285.1	(17)	172.2	(23)	296.3	(17)
m-SPC Pigmented	194.7	(15)	111.6	(24)	244.9	(16)	106.7	(22)	161.5	(16)

* = Total counts

TABLE 24. GEOMETRIC MEAN BACTERIAL CONCENTRATIONS (#/ml) IN THE GARDEN GROVE WATER DISTRIBUTION SOURCE & SITE LOCATIONS

Plate Count Technique	Source (N)	Sites Sandra #2	Rockinghorse	Harris	Marshall
Total Plate Count Medium 35°C Spread Plate	159.7 (40)	442.1	503.6	1479.5	511.3
Total Plate Count Pigmented 35°C Spread Plate	61.1 (40)	98.8	148.3	434.1	184.3
Total Plate Count Medium 35°C Pour Plate	119.9 (41)	281.7	319.0	1026.7	257.1
Total Plate Count Pigmented 35°C Pour Plate	21.5 (41)	7.5	20.1	26.7	70.3
Total Plate Count Medium 20°C Spread Plate	120.7 (40)	550.9	513.7	1368.0	522.5
Total Plate Count Pigmented 20°C Spread Plate	36.4 (40)	121.4	65.4	1010.6	120.1
Total Plate Count Medium 20°C Pour Plate	124.7 (20)	337.2	419.7	803.7	509.3
Total Plate Count Pigmented 20°C Pour Plate	13.7 (20)	30.9	29.3	215.3	48.5
Low Nutrient R-2A Medium	260.7 (40)	1507.9	976.2	1546.2	710.2
Low Nutrient R-2A Pigmented	108.7 (40)	195.5	160.2	935.9	34.2
m-Standard Plate Count Total	467.8 (39)	1607.8	992.6	3273.5	1162.4
m-Standard Plate Count Pigmented	236.4 (38)	423.9	273.4	2019.1	378.8

TABLE 25 MONTHLY MEAN BACTERIAL CONCENTRATIONS (#/ml) IN THE GARDEN GROVE WATER DISTRIBUTION SYSTEM

	TPC35SP				TPCP35SP				TPC35PP				TPCP35PP			
	Sources	(N)	Sites	(N)	Sources	(N)	Sites	(N)	Sources	(N)	Sites	(N)	Sources	(N)	Sites	(N)
7/78	-		1000.0	(1)	-		-		-		-		-		-	
8/78	-		172.5	(8)	-		-		-		-		-		-	
9/78	-		1764.6	(10)	-		-		-		-		-		-	
10/78	-		895.7	(9)	-		586.1	(9)	-		-		-		-	
11/78	-		697.5	(8)	-		266.0	(8)	-		545.0	(4)	-		42.5	(4)
12/78	-		598.8	(8)	-		186.2	(8)	-		354.3	(7)	-		41.3	(4)
1/79	210.0	(2)	379.6	(7)	115.0	(2)	127.0	(7)	165.0	(2)	363.6	(7)	17.5	(2)	18.3	(7)
2/79	-		305.4	(8)	-		84.0	(8)	-		348.5	(8)	-		17.3	(8)
3/79	-		477.9	(8)	-		372.3	(8)	-		225.4	(8)	-		20.3	(8)
4/79	154.5	(6)	211.8	(12)	81.8	(6)	56.7	(12)	189.5	(6)	317.8	(12)	5.8	(6)	13.3	(12)
5/79	140.0	(1)	314.4	(8)	0.0	(1)	146.9	(8)	142.5	(2)	295.0	(8)	0.0	(2)	12.5	(8)
6/79	830.0	(2)	901.3	(8)	252.5	(2)	266.9	(8)	650.0	(2)	231.4	(7)	32.5	(2)	6.4	(7)
7/79	120.0	(2)	873.1	(7)	78.5	(2)	281.7	(7)	105.0	(2)	236.4	(7)	25.0	(2)	1.4	(7)
8/79	46.7	(3)	294.1	(11)	10.0	(3)	137.3	(11)	28.3	(3)	156.4	(11)	0.0	(3)	22.3	(11)
9/79	426.7	(3)	795.6	(8)	266.7	(3)	270.0	(8)	321.7	(3)	1104.4	(8)	218.3	(3)	189.4	(8)
10/79	34.3	(3)	2054.5	(4)	13.3	(3)	131.0	(4)	23.3	(3)	1686.0	(4)	5.0	(3)	21.3	(4)
11/79	33.3	(3)	4942.5	(2)	0.0	(3)	5.0	(2)	21.7	(3)	1337.5	(2)	0.0	(3)	0.0	(2)
12/79	113.3	(3)	527.5	(2)	15.0	(3)	0.0	(2)	21.7	(3)	392.5	(2)	5.0	(3)	0.0	(2)
1/80	126.0	(3)	1131.0	(2)	5.0	(3)	268.0	(2)	38.3	(3)	602.5	(2)	0.0	(3)	0.0	(2)
2/80	83.0	(3)	1167.5	(2)	3.3	(3)	1035.0	(2)	13.3	(3)	930.0	(2)	0.0	(3)	0.0	(2)
3/80	125.7	(3)	221.5	(2)	36.7	(3)	5.0	(2)	59.7	(3)	157.5	(2)	3.3	(3)	0.0	(2)
4/80	11.3	(3)	750.0	(2)	3.3	(3)	0.0	(2)	23.3	(3)	595.0	(2)	0.0	(3)	0.0	(2)

TABLE 26 MONTHLY MEAN BACTERIAL CONCENTRATIONS (#/ml) IN THE IRVINE RANCH WATER DISTRIBUTION SYSTEM

	TPC35SP		TPCP35SP		TPC35PP		TPCP35PP	
	Sources (N)	Sites (N)	Sources (N)	Sites (N)	Sources (N)	Sites (N)	Sources (N)	Sites (N)
6/78	-	4214.0 (5)	-	-	-	-	-	-
7/78	-	567.6 (5)	-	-	-	-	-	-
8/78	-	58.3 (9)	-	-	-	-	-	-
9/78	-	571.5 (8)	-	-	-	-	-	-
10/78	-	114.8 (9)	-	35.4 (9)	-	-	-	-
11/78	-	78.1 (8)	-	14.4 (8)	-	91.3 (4)	-	15.0 (4)
12/78	-	246.4 (7)	-	25.0 (7)	-	163.6 (7)	-	2.0 (7)
1/79	315.0 (3)	103.2 (10)	1.7 (3)	21.6 (10)	65.0 (3)	72.3 (10)	0.0 (3)	15.2 (10)
2/79	-	371.1 (8)	-	48.1 (8)	-	129.8 (8)	-	27.4 (8)
3/79	25.0 (1)	130.8 (8)	20.1 (1)	14.8 (8)	5.0 (1)	169.4 (7)	1.0 (1)	21.0 (7)
4/79	22.5 (2)	181.3 (12)	0.0 (2)	31.1 (12)	9.0 (2)	140.1 (12)	3.0 (2)	17.6 (12)
5/79	25.0 (1)	261.3 (8)	0.0 (1)	27.5 (8)	10.0 (1)	288.4 (8)	0.0 (1)	61.8 (8)
6/79	25.0 (1)	82.5 (8)	5.0 (1)	41.3 (8)	10.0 (1)	47.1 (7)	0.0 (1)	7.1 (7)
7/79	3.0 (1)	152.5 (8)	0.0 (1)	45.6 (8)	-	44.4 (8)	-	0.0 (8)
8/79	40.0 (1)	97.5 (12)	0.0 (1)	27.5 (12)	30.0 (1)	77.5 (12)	0.0 (1)	16.7 (12)
9/79	5.0 (1)	122.5 (8)	0.0 (1)	36.9 (8)	25.0 (1)	28.8 (8)	0.0 (1)	12.5 (8)
10/79	30.0 (1)	125.0 (4)	5.0 (1)	85.0 (4)	21.0 (1)	73.3 (4)	0.0 (1)	26.3 (4)
11/79	30.0 (1)	240.0 (2)	15.0 (1)	15.0 (2)	20.0 (1)	155.0 (2)	0.0 (1)	2.5 (2)
12/79	10.0 (1)	240.0 (2)	0.0 (1)	0.0 (2)	20.0 (1)	37.5 (2)	5.0 (1)	2.5 (2)
1/80	14.0 (1)	355.0 (2)	7.0 (1)	35.0 (2)	0.0 (1)	62.5 (2)	0.0 (1)	5.0 (2)
2/80	40.0 (1)	40.0 (1)	30.0 (1)	5.0 (1)	40.0 (1)	10.0 (1)	0.0 (1)	0.0 (1)
3/80	23.0 (1)	65.0 (2)	10.0 (1)	15.0 (2)	10.0 (1)	35.0 (2)	0.0 (1)	0.0 (2)
4/80	40.0 (1)	185.0 (2)	0.0 (1)	5.0 (2)	30.0 (1)	110.0 (2)	0.0 (1)	0.0 (2)

high counts versus the significance of bacterial genera present in the water distribution system. Further studies must be undertaken to resolve these two important questions.

Seasonal Evaluation of Bacterial Numbers

Seasonal changes in bacterial numbers can be caused by changes in physical or chemical factors in a water distribution system. However, data contained in Appendix A (Tables A3-A16) indicate that for the parameters examined the two systems in this study were relatively constant over time. Only water temperature showed a significant seasonal fluctuation. Monthly examinations of the mean total bacterial numbers and pigmented counts for both the Garden Grove and Irvine Systems using TPC35SP and TPC35PP methods revealed no discernable seasonal trends (Tables 25 and 26). Furthermore, no seasonal patterns were noted when the magnitude of increase or decrease in total monthly mean counts from source(s) to sites were examined during the period of January through April, 1980.

Physical and Chemical Parameters and Bacterial Counts

The free residual chlorine concentration correlated negatively with bacterial numbers for all bacterial media and plating techniques employed. Total counts on TPC35SP, TPC35PP, TPC20PP and R-2A media were significant at $p \leq 0.01$ and r values ranged between -0.2 and -0.3. No significant correlations were noted between any of the six physical factors and pigmented bacterial counts on all media or total bacterial counts on m-SPC.

Nitrate, total cations, total anions, calcium, magnesium, sodium and potassium results correlated with bacterial counts on a number of media in Irvine and Garden Grove (Table 27). Interestingly, negative relationships for these variables were found for the Irvine samples and positive relationships were found for the Garden Grove samples. In the Irvine System m-SPC medium showed no correlations with any of these factors, and only with magnesium in the Garden Grove system.

These variables showed the best correlations in Garden Grove with R-2A and TPC20PP followed by TPC20SP. Pigmented bacterial counts did not correlate with any of these factors. Pigmented bacteria appeared to be less affected by chemical and physical factors than did nonpigmented bacteria. These data suggest that the pigmented group of bacteria should be examined in greater detail regarding nutritional requirements and resistance to stressful environments.

Because of the negative correlations between bacterial counts and the inorganic parameters listed above for the Irvine System
residual chlorine may be the most important factor in determining total bacterial counts in this system. However, it does not adequately explain fluctuations in bacterial counts in the system.

Particulates, Particle Size and Bacterial Numbers

For the Irvine Ranch Water Distribution System sites, no correlative relationship was found between the total number of particles per milliliter

TABLE 27. PEARSON'S r CORRELATION COEFFICIENTS BETWEEN CHEMICAL PARAMETERS AND BACTERIAL COUNTS ON TEST MEDIA

Media	Total Cations	Total Anions	Calcium	Mg	Na	K	NO_3
Garden Grove							
TPC35SP	.371[1] (58)	NS	.39 (58)	NS	NS	.28 (58)	NS
TPC35PP	.38 (59)	NS	.41 (59)	NS	NS	.28 (59)	NS
TPC20SP	.36 (56)	NS	.41 (56)	.37 (56)	NS	.31 (56)	NS
TPC20PP	.57 (32)	NS	.64 (32)	.57 (32)	.37 (32)	.33 (32)	NS
R-2A	.42 (56)	NS	.46 (56)	.31 (56)	.26 (56)	.29 (56)	NS
m-SPC	NS	NS	NS	NS	NS	NS	NS
Irvine							
TPC35SP	NS	NS	NS	NS	NS	NS	.45 (29)
TPCP35PP	NS	NS	NS	.33 (29)	NS	NS	NS

(continued)

Table 27 Continued

	Total Cations	Total Anions	Calcium	Mg	Na	K	NO_3
TPC20SP	NS	NS	NS	NS	NS	NS	.33 (28)
TPC20PP	NS	NS	NS	NS	-.39 (22)	.37 (22)	.37 (22)
TPCP20PP	NS	NS	NS	NS	.40 (21)	NS	.38 (22)
R-2AP	NS	.40 (27)	NS	NS	NS	-.39 (27)	NS
mSPC	.50 (13)	NS	NS	NS	NS	NS	NS

NS = not significant at $\alpha < 0.05$
[1] $r > 0.25$ significant at $\alpha < 0.05$

and total or pigmented bacterial counts ($n = 14$, $p \leq .001$). Analysis of the source water data yielded only one correlation between Irvine source water total particle counts and media (TPC35PP, $r = 0.99$, $n = 14$, $p \leq 0.00$).

However, for sites in the Garden Grove System, bacterial count results obtained on a number of media covaried similarly with total particle counts (Table 28). The site data in Table 28 indicate that as particulate counts increased so did bacterial numbers. This was verified, in a qualitative sense, by scanning electron microscope (SEM) examinations of water samples which showed that bacteria were associated with particles in samples that had higher particulate counts (see section on Scanning Electron Microscopy). Particles with bacteria associated have been recovered from the Irvine Water Distribution System. These findings suggest the importance of particles and the chemical nature of these particles in influencing the bacteriological quality of water systems. In Tables 9 and 12, the data indicate that both Garden Grove and Irvine particle and bacterial counts increased from source to sample site location. The distribution of particle sizes remained similar from source to site in each system (Table 9). The similarity of particle size distribution data and the occurrence of bacteria associated with particulates suggest that the chemical nature of the particles, the residence time of the particle in the system, and the pressure maintained in the distribution system are all important factors in determining and understanding the relationship between particles and bacterial numbers in water distribution systems.

Certain particle size ranges were correlated with bacterial numbers in the Garden Grove System. Interestingly, although correlations between low nutrient R-2A medium and total particles at the source were not found, there was an association between the low nutrient R-2A medium and two particle size ranges in Garden Grove source water. Pigmented counts were correlated with $> 20\text{-}25\ \mu m$ ($r = 0.33$, $n = 34$, $p \leq 0.05$) and total pigmented counts correlated with $> 70\text{-}100\ \mu m$ ($r = 0.45$, $r = 0.47$, respectively; $n = 34$, $p \leq 0.05$). Only two other media, TPC35PP and TPC20PP showed any relationship at $p \leq 0.05$ and both occurred within the 40-50 μm size range.

Negative correlations ($p = 0.05$) were found between most media and particles in the 2.5-7 μm size range for the Garden Grove system. This trend changed dramatically as particle size increased. The data clearly indicated strong correlative relationships among media and particle sizes ranging from 15-70 μm. This suggests that particles may be a vehicle for moving bacteria through the distribution system and that disruption may occur when larger particles of bacteria are processed by standard methods, thus increasing bacterial counts. These data also agree with scanning electron microscopy information presented later.

In the Irvine source water relatively few correlations between media and particle size were evident. No discernable trend was apparent except that negative relationships were demonstrated. Site data showed also a paucity of relationships with only four significant correlations occurring among all the media in the 2.5-20 μm size ranges and no relationships in the size ranges of $> 20\text{-}100\ \mu m$. TPC35TP and TPC35PP covaried positively with the 100-150 μm size range ($r = 0.46$ and 0.40, respectively, $n = 23$, $p \leq 0.05$).

TABLE 28. PEARSON'S r BETWEEN BACTERIAL NUMBERS ON TEST MEDIA AND TOTAL PARTICLES/ML IN THE GARDEN GROVE DISTRIBUTION SYSTEM

	Source		Sites	
	r	(N)	r	(N)
TPC35SP	NS*	(34)	NS	(24)
TPCP35SP	NS	(34)	NS	(24)
mSPC	NS	(33)	NS	(23)
mSPCP	NS	(33)	.40	(23)
TPC35PP	NS	(35)	.51	(24)
TPCP35PP	NS	(35)	NS	(24)
TPC20SP	NS	(34)	.40	(24)
TPCP20SP	NS	(34)	.40	(24)
TPC20PP	NS	(20)	NS	(13)
TPCP20PP	NS	(20)	.62	(13)
Low Nutrient R-2A	NS	(34)	.50	(23)
Low Nutrient R-2A Pigmented	NS	(34)	.40	(23)

TPC35SP = Total Plate Count Medium 35°C Spread Plate
TPCP35SP = Total Plate Count Pigmented 35°C Spread Plate
TPC35PP = Total Plate Count Medium 35°C Pour Plate
TPCP35PP = Total Plate Count Pigmented 35°C Pour Plate
TPC20SP = Total Plate Count Medium 20°C Spread Plate
TPCP20SP = Total Plate Count Pigmented 20°C Spread Plate
TPC20PP = Total Plate Count Medium 20°C Pour Plate
TPCP20PP = Total Plate Count Pigmented 20°C Pour Plate
mSPC = m-Standard Plate Count Medium Total Count
mSPCP = m-Standard Plate Count Medium Pigmented Count
(N) = number of samples
Level of significance ≤ 0.05 *NS = not significant

Comparison of results from the two systems suggests that particles are a better predictor of bacterial counts in the unchlorinated system than the chlorinated system. Yet SEM evidence indicated colonized particles in both systems. Lack of correlative relationships in the chlorinated system may indicate that disinfection has inactivated bacteria. However, increases in bacterial counts still occurred between source and site locations, demonstrating that a complex situation exists.

Bacterial Identifications

Chlorinated system. The majority of bacteria isolated from the chlorinated system were gram-negative rods. At the chlorinated source location *Pseudomonas*/ *Alcaligenes*, *Acinetobacter* and *Flavobacterium* were repeatedly recovered from the water (Figure 7). In January 1979, the dominant genera were *Acinetobacter* (46%) and *Flavobacterium* (41.6%). A similar distribution occurred during January 1980 when *Acinetobacter* represented 34% of the population and *Flavobacterium* 34.8%. These data suggested a seasonal predominance of these genera. The occurrence of *Flavobacterium* decreased dramatically by the next sampling period, April 1979, and then slowly increased reaching maximal numbers again in January 1980. This genus was not isolated during April 1980 sampling. In April 1979 bacteria identified as non-lactose fermenting *Klebsiella* represented the greatest proportion of the population (57.0%). *Pseudomonas/Alcaligenes* became the predominant bacterial group only during July 1979 (60.7%) and this occurrence coincided with a decrease in *Acinetobacter* and *Flavobacterium* genera. During April 1980, a small proportion of gram-positive bacteria were detected and *Acinetobacter* became the predominant genus followed by the *Pseudomonas/Alcaligenes* group. Other bacterial genera isolated during the course of the study included *Pseudomonas*, *Pasteurella*, *Serratia* and *Enterobacter*.

At the chlorinated site location (Figure 8) *Flavobacterium* was the predominant genus in the population during September 1978 (26.7%), January 1979 (49.8%), April 1979 (93.0%) and October (94.7%). The dominant group found during July 1979, January 1980 and April 1980, was *Pseudomonas/Alcaligenes* which accounted for 83.9%, 77.4% and 95.5% of the isolated population, respectively. At least five other genera (Figure 8), *Pseudomonas* sp., *Pasteurella* sp., *Serratia* sp., *Enterobacter* sp., and *Klebsiella* sp., were isolated during at least one sampling time, but never represented more than 10% of the total bacterial population. *Acinetobacter* was identified in four of the seven sampling periods and when present comprised between one and 30% of the bacterial population. Gram-positive cocci were isolated only during April 1980. Some strains have been identified as *Staphylococcus saprophyticus* (Dr. B. Austin, Ministry of Agriculture, Fisheries & Food, Dorset, England, personal communication, 1980).

These data for the chlorinated system suggest that although a number of different genera were isolated, only one to three generally contributed significantly to the total population at a given time (Figures 7 and 8). Predominance was usually distributed among several genera at the source narrowing to a single genus or group at the site location at each sampling time. The predominant bacterial genera change between source and site location suggesting that chemical or physical factors in the distribution line strongly in-

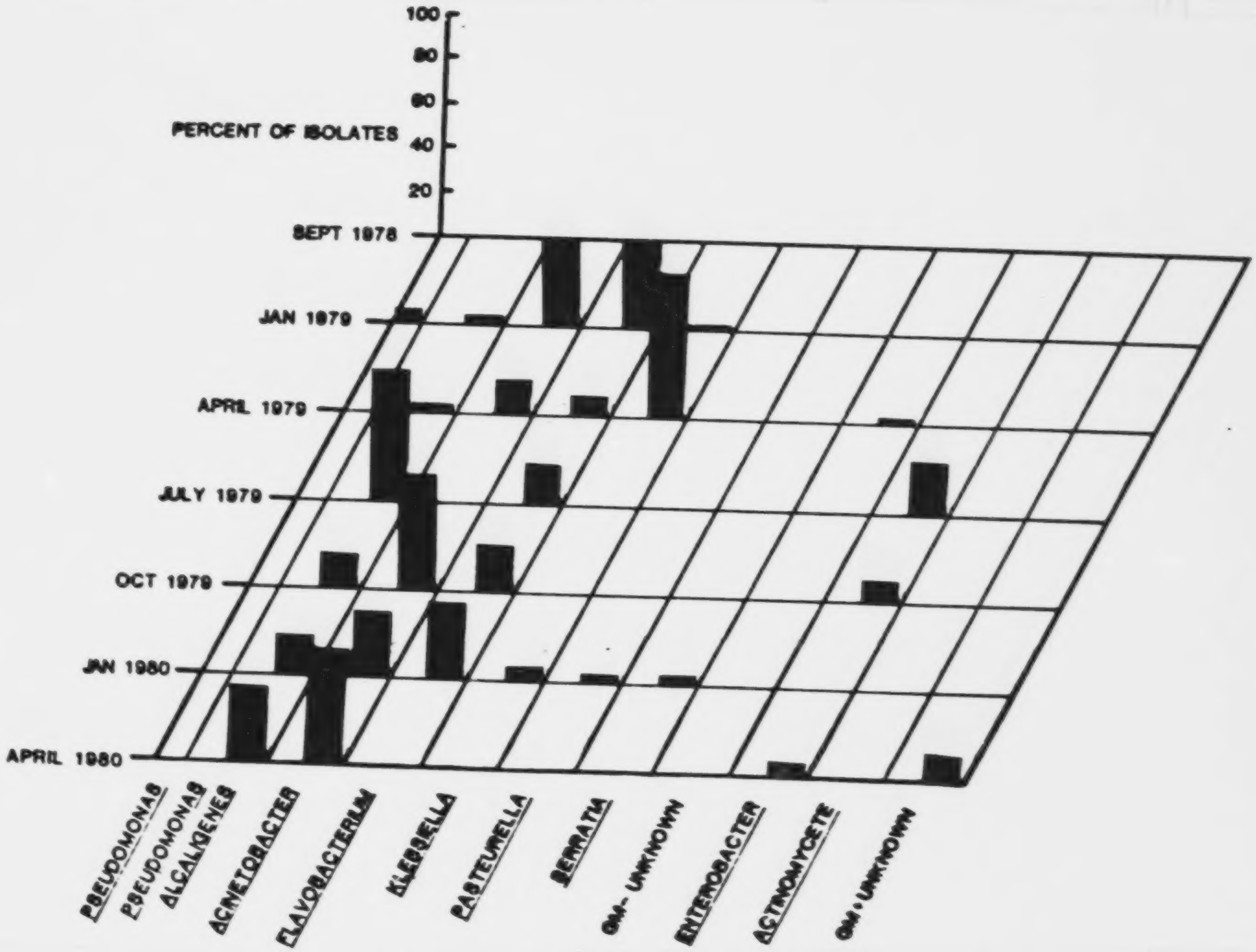

Figure 7. Bacterial Genera Represented at Irvine Source at the Designated Sampling Intervals.

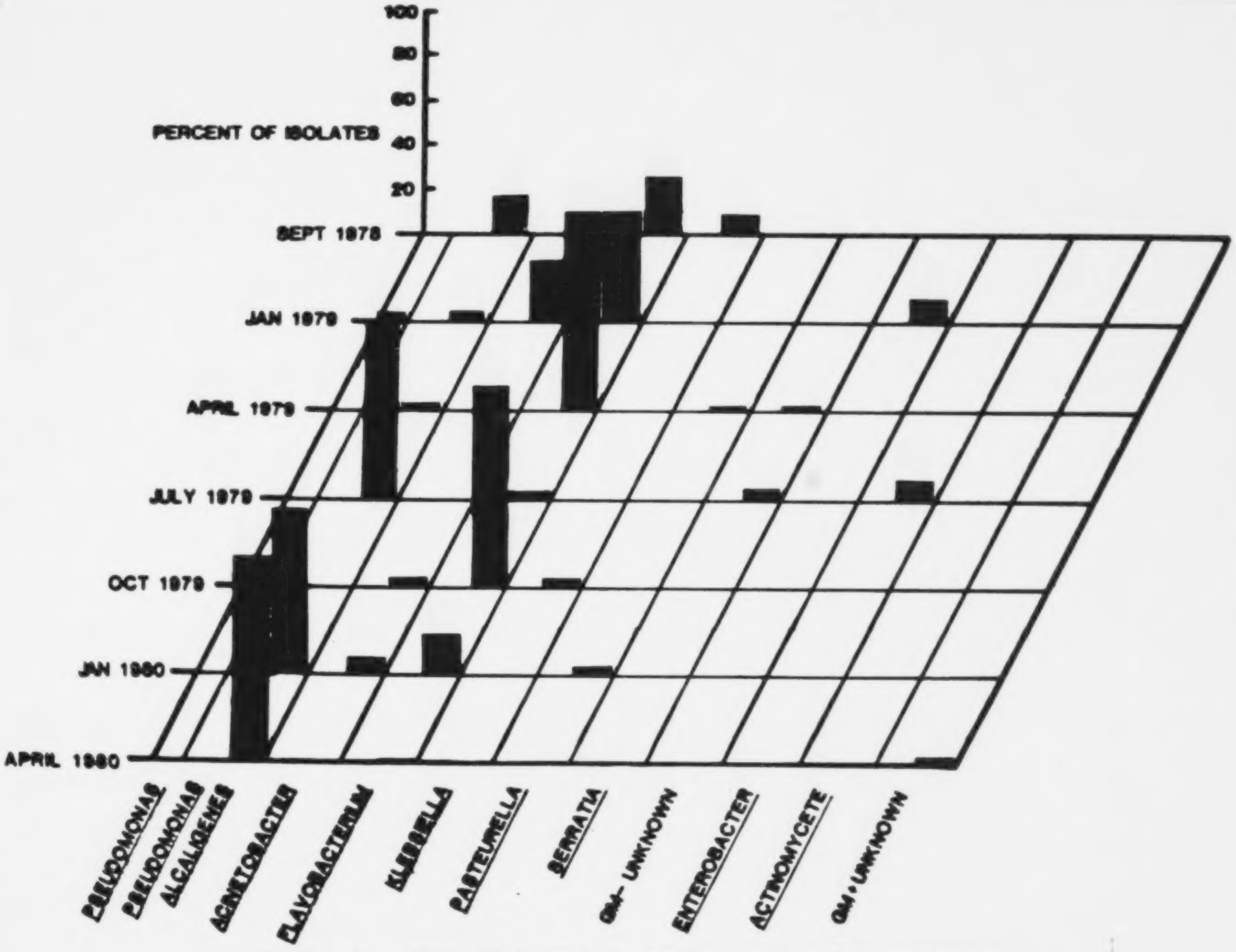

Figure 8. Bacterial Genera Represented at Paseo Picasso at the Designated Sampling Intervals.

fluence establishment and growth of certain bacterial groups through the water main.

Unchlorinated system. The source water for the site location in the unchlorinated system was composed of three wells. The influence that each well had on the composition of the water reaching the site varied throughout the study depending on pumping regimes and was evident by the composition of the bacterial populations among the well sources and site location.

At well 6064, which represented a small portion of the water entering the distribution system, it was noted that Acintobacter, Pseudomonas/Alcaligenes and Flavobacterium were the predominant genera (Figure 9). During July 1979 a large population of gram-positive organisms was detected (45.7%). Gram-positive cocci were again identified as Staphylococcus saprophyticus (Dr. B. Austin, personal communication). Pseudomonas/Alcaligenes organisms were found in increased numbers with each sampling time until October 1979; subsequently, this group also represented a substantial proportion of the population during January and April 1980.

Four major groups of bacteria were repeatedly isolated at well 6321 and each was dominant at a particular sampling period, except during October 1979 (Figure 10). The genera included Klebsiella (53.6% during January 1979), Pseudomonas/Alcaligenes (66.0% during April 1979), Flavobacterium (57.1% during July 1979), and Acinetobacter (56.8% during January and April 1979, but after these dates were undetected for the remainder of the study). Gram-positive unknown bacteria were infrequent in occurrence, but represented 94.9% of the bacterial population in April 1980. The majority of these isolates were gram-positive cocci.

Well 23/9 was included in the study as of October 1979, in order to more clearly define the chemical parameters at the site location (Figure 11). Flavobacterium represented 27.8% of the bacterial population during October 1979, and increased at the next sampling in January 1980 (43.1%) (Figure 11). The Pseudomonas/Alcaligenes group and Acinetobacter represented part of the population during the three sampling periods. A significant percentage of gram-positive bacteria were also detected at this well throughout the investigation and were preliminarily classified as gram-positive cocci.

At the unchlorinated site location a diverse bacterial population was recorded during September 1978 (Figure 12). During this sampling period, Acinetobacter represented 42% of the total population. After this time it was continually recovered from the site location, but in small numbers. In January 1979, a large number of gram-positive organisms were isolated. Concurrently the gram-negative proportion of the population decreased, but still represented a diverse number of genera. During each sampling period Pseudomonas/Alcaligenes were isolated and remained the major genera isolated from this site. The percentage of the population represented by Pseudomonas/Alcaligenes during April 1979, July 1979, October 1979, January 1980 and April 1980 was: 71.4%, 59.5%, 41.4%, 80.3% and 38.5%, respectively. In addition, small but persistent numbers of Acinetobacter, Flavobacterium and Klebsiella organisms were detected at this site. The gram-positive unknown proportion of the population reached its peak at the site location during the month of

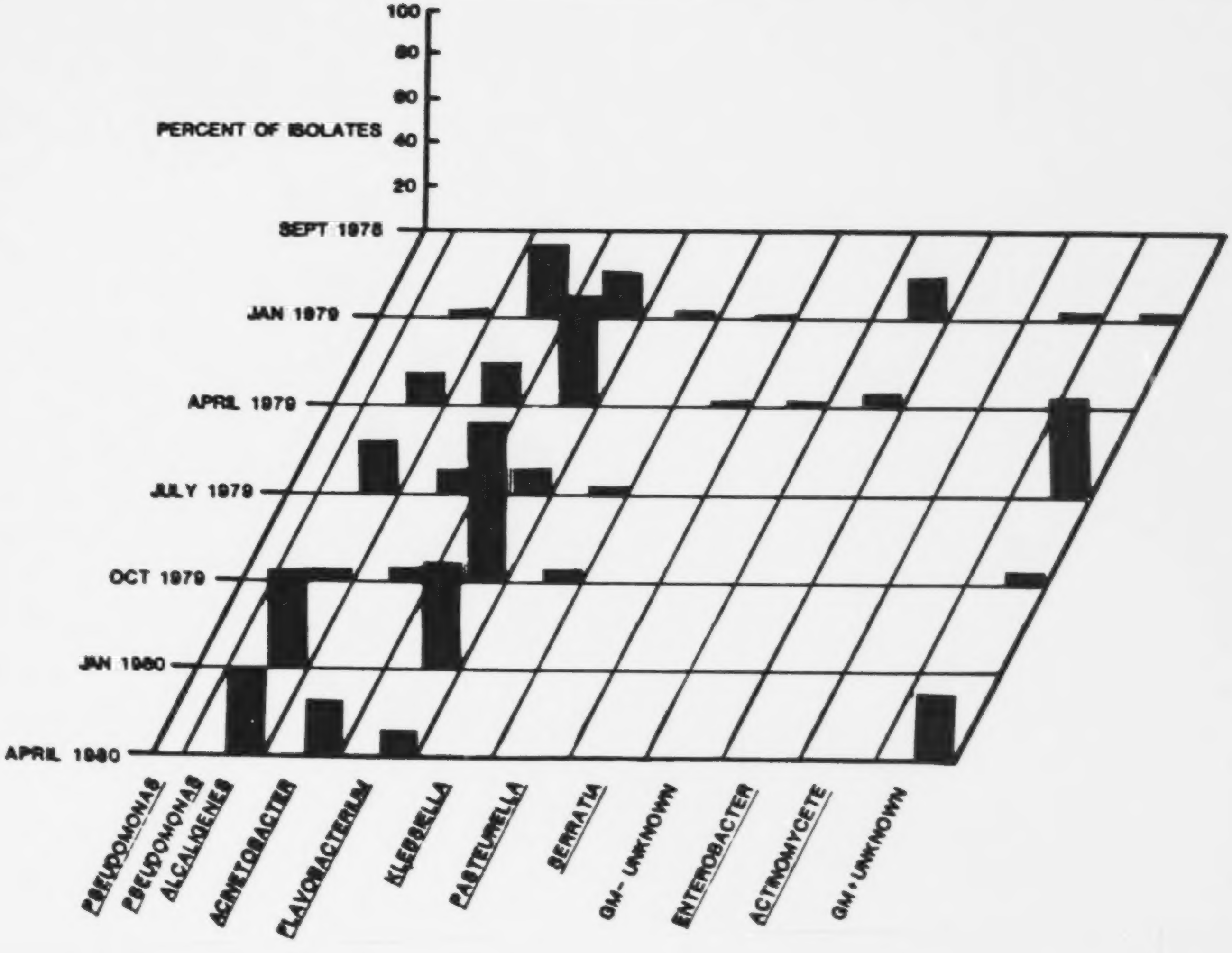

Figure 9. Bacterial Genera Represented at Well 6064 at the Designated Sampling Intervals.

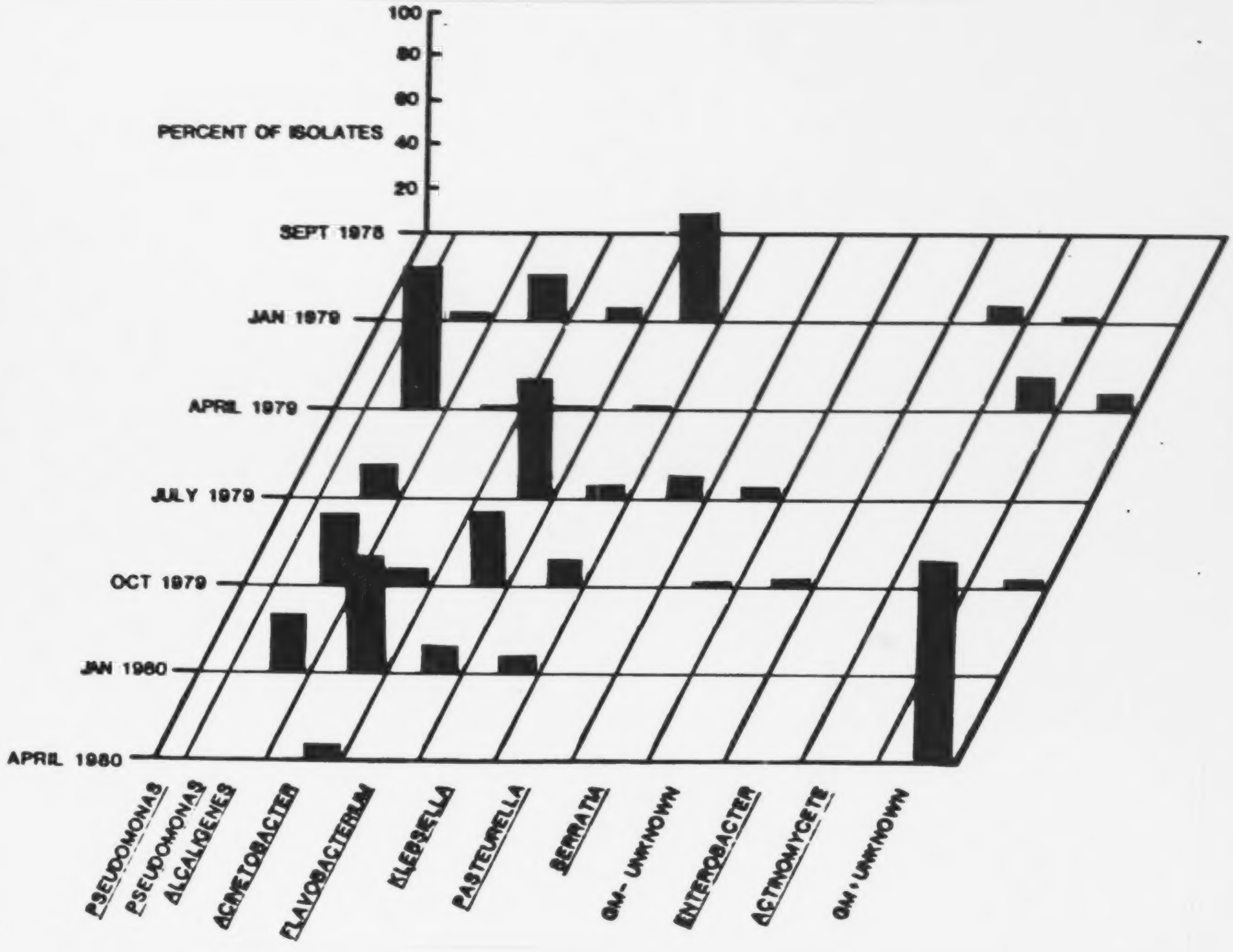

Figure 10. Bacterial Genera Represented at Well 6321 at the Designated Sampling Intervals.

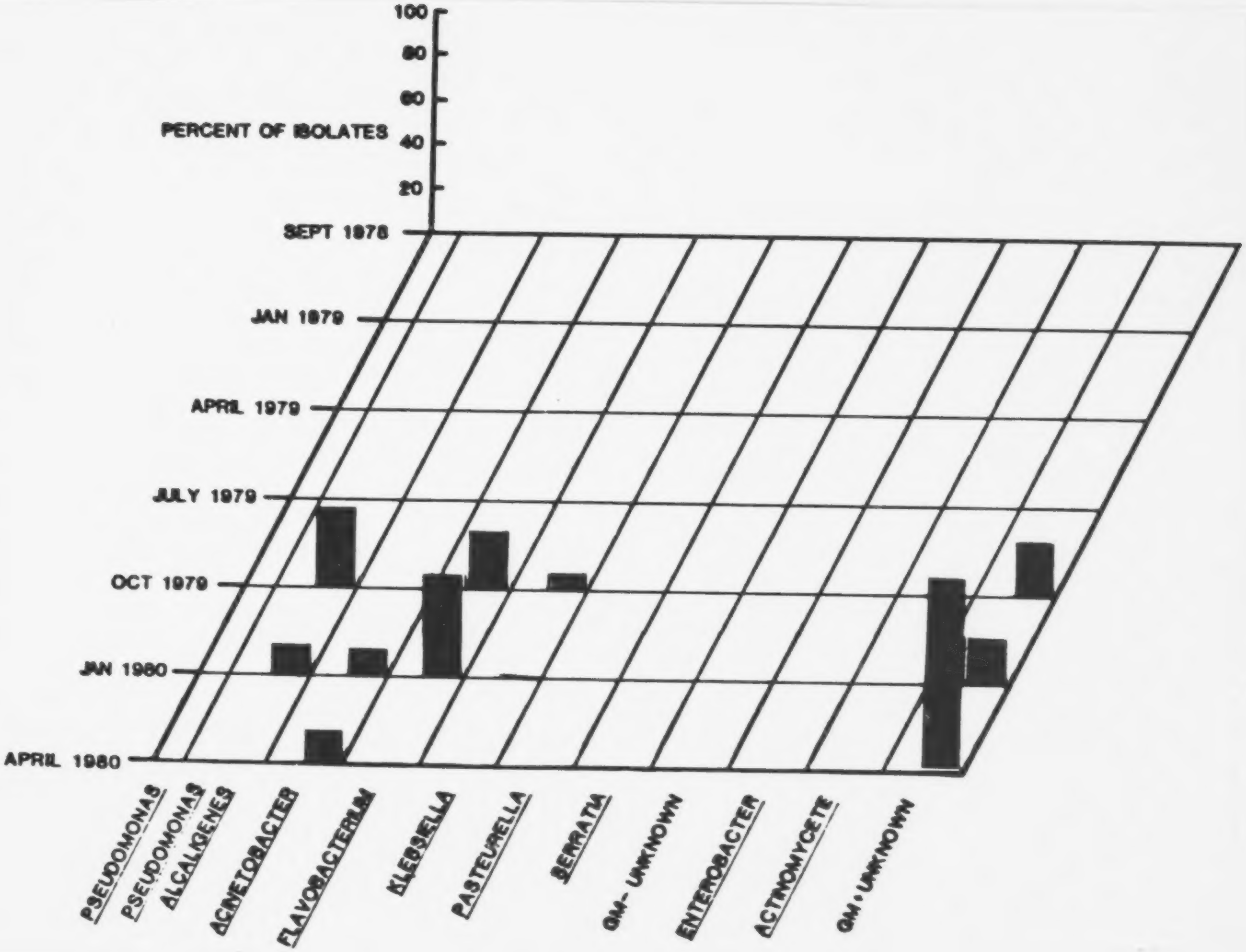

Figure 11. Bacterial Genera Represented at Well 23/9 at the Designated Sampling Intervals

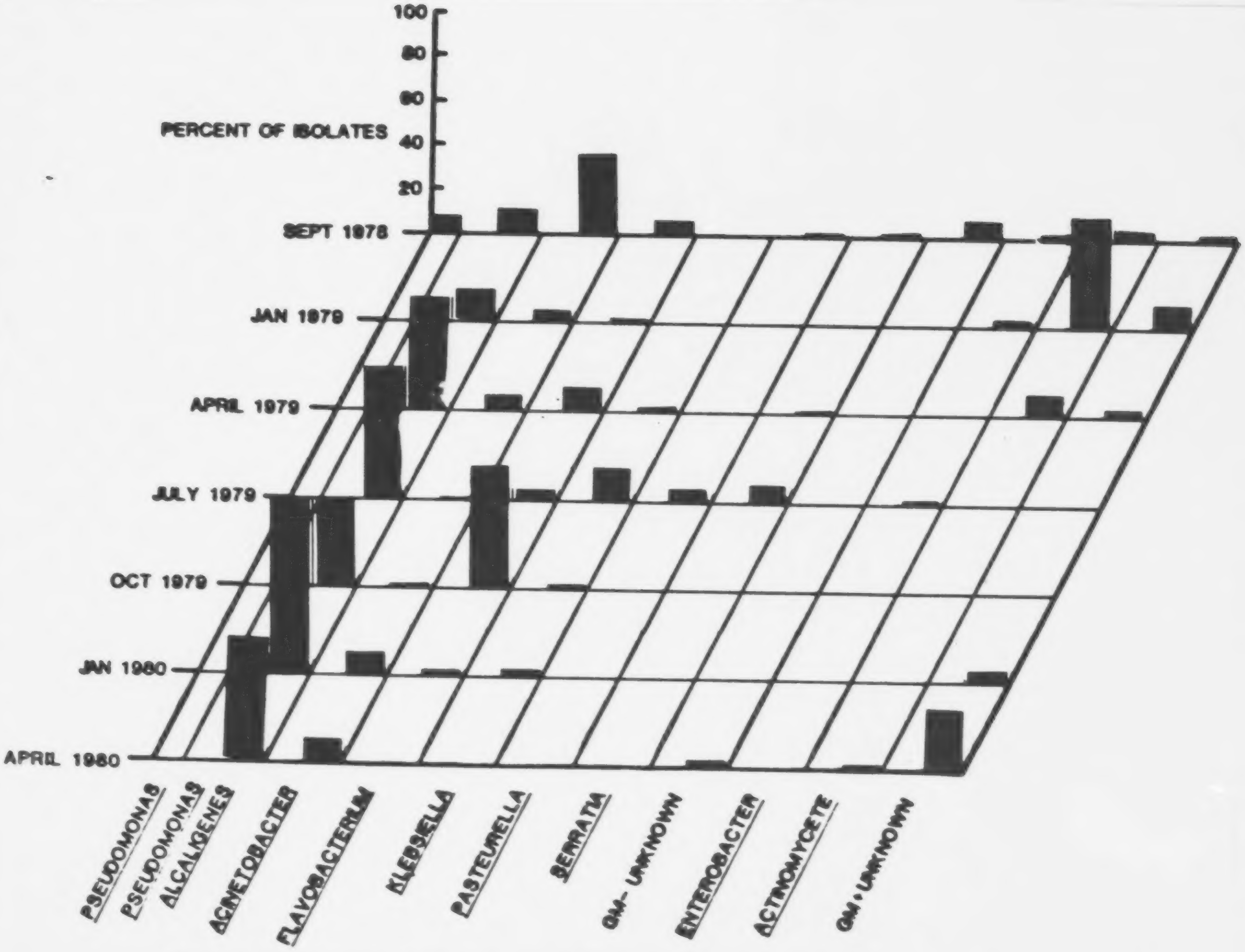

Figure 12. Bacterial Genera Represented at Rockinghorse at the Designated Sampling Intervals.

January 1979, then decreased significantly throughout the remainder of that year and were not detected again until January and April 1980. During April of 1979 this proportion of the population represented 30.6%. Pasteurella, Serratia and Enterobacter were isolated from plate count agar on two to three sampling occasions at this site.

Comparing the unchlorinated sources to site, the diversity of bacteria at the site appeared to come from a combination of the three sources. The distribution of bacterial genera found at well 6321 were most closely related to those recovered from the site. The data from the unchlorinated system indicated that possible seasonal variation of particular bacteria genera did occur. As in the chlorinated system the Acinetobacter organisms either were not detected or appeared in small numbers during the warmer months. This phenomenon was most evident at well 6321. In addition the gram-positive population decreased significantly during July 1979, except at well 6064. Flavobacterium was detected at the site and all sources on most sampling dates indicating its stability in the potable water system.

Coliforms

Only a small proportion of the total bacterial population identified from plate count media were coliform organisms which were isolated periodically. In the chlorinated system Enterobacter sp. were detected at the Irvine site during October 1979 and represented 1.4% and 4.8% of the isolates, respectively. Standard coliform testing produced positive results in the chlorinated system during the months of June, July, August and November 1979, and January 1980. In the unchlorinated system positive results occurred in April and June through November 1979. Enterobacter cloacae was the predominant isolate from the Irvine system using the most-probable-number method and represented 83.3% of the isolated coliforms. Lactose fermenting Klebsiella represented 77.8% of the coliforms isolated in the unchlorinated system. Isolation of coliforms from plant count agar did not necessarily correspond with positive total coliform results by conventional procedures.

Influence of Selected Chemical and Physical Parameters

For the seven chemical parameters examined no statistically significant differences were found between source(s) and site data (Table 29). The data presented in Table 29 indicate that turbidity and total particle counts increased between source(s) and site locations in both systems. Further, as would be expected, the free chlorine residual at the well sites in the unchlorinated system was negligible but because of water supplementation practices the site location showed a small free chlorine residual on a seasonal basis.

Total organic carbon appears to be too gross a parameter to assess nutrient changes that would affect the genera composition of the bacterial populations identified in this study. Therefore, more specific nutrient parameters are required. Based on the types of bacterial genera found in the two systems, amino acid analysis of water may provide a mechanism for predicting changes in bacterial population composition.

TABLE 29. MEAN VALUES FOR PHYSICAL AND CHEMICAL PARAMETERS IN THE WATER DISTRIBUTION SYSTEMS

Chlorinated IRWD System

Constituents	Source	Site
Conductivity (μmhos)	894	892
pH	7.8	7.7
Turbidity (NTU)	0.42	2.3
Dissolved Oxygen (mg/l)	9.7	9.2
Free chlorine Residual (mg/l)	0.73	0.60
Total Organic Carbon (mg/l)	2.70	3.06
Total Particles	241	1463

Unchlorinated Garden Grove System

Constituents	Well 6064	Well 6321	Well 23/9	Site
Conductivity (μmhos)	705	650	800	855
pH	7.2	7.4	6.9	7.4
Turbidity (NTU)	0.27	0.23	0.3	5.0
Dissolved Oxygen (mg/l)	7.7	7.4	7.9	7.1
Free Chlorine Residual (mg/l)	0.0	0.01	0.0	0.4
Total Organic Carbon (mg/l)	0.5	0.5	0.5	1.0
Total Particles	30	44	304	1090

Differences in the occurrence of bacterial genera can be explained by all or a combination of the following three hypotheses. 1) The distribution systems operated in a slug flow manner. Thus, each source and site sample represented a different water. 2) Chemical parameters not measured in this study such as amino acid concentrations could be controlling factors which seasonally influence the composition of the bacterial populations through available nutrients. 3) Bacterial microcolonies along pipe surfaces could sporadically slough off or shed bacteria or bacterially colonized particles into the system at random intervals causing unpredictable counts. Micronutrient availability and microhabitats in distribution systems are still poorly understood. Recent works have shown a diverse number of habitats in pipe systems (4, 106), but the suitability for bacterial colonization and factors that influence bacterial colonization are not yet delineated.

Fungal Enumeration

The frequency of fungal colonies and species (initial estimate) observed over the period of the study at each sampling site is indicated in Table 30. The Irvine system, despite chlorination, exhibited a mean fungal colony level almost twice that of the unchlorinated Garden Grove system. Interestingly also, the mean number of fungal colonies and species decreased from winter to summer. Unfortunately, due to the large standard deviations, differences in means were not statistically significant. Seasonal and overall total results are shown for each distribution system.

Correlations between the numbers of fungal colonies and the other parameters in the study are indicated in Table 31. The corresponding correlations for frequency of fungal species are shown in Table 32. In Tables 31 and 32 only correlations over ± 0.50 are indicated, and only variables with significant correlations are shown.

Correlations of Fungal Colony Counts with Physical and Chemical Parameters

In the Irvine system, of the physical parameters (residual chlorine, temperature, pH, redox potential, electroconductivity and turbidity), only pH correlated significantly with fungal colony counts ($r = 0.61$). Total and pigmented counts from all media except m-SPC (which was not tested) did not correlate with fungal counts.

In the Garden Grove system, none of the physical or bacteriological parameters correlated significantly with fungal colony counts. As indicated by Table 31, differences were obtained between overall winter, spring and summer samples. For winter samples, turbidity and pH correlated significantly with fungal counts, but for spring and summer samples, a significant correlation was absent.

Combined data for both distribution systems and all three seasons indicate that of all the physical and bacteriological parameters, only pH gave a significant positive correlation with fungal colony counts ($r = 0.47$, $n = 56$).

TABLE 30. FREQUENCY OF FUNGAL COLONIES AND FUNGAL SPECIES (#/100 ml) IN IRVINE AND GARDEN GROVE WATER DISTRIBUTION SYSTEMS

	WINTER		SPRING		SUMMER	
	Frequency of		Frequency of		Frequency of	
Location	fungal colonies	fungal species	fungal colonies	fungal species	fungal colonies	fungal species
Irvine						
Paseo Picasso	7.5	7.5	1.0	1.0	22.0	9.0
Via Messina	12.5	12.5	13.0	6.0	2.0	2.0
Arbor Lane	483.0	20.0	15.0	5.0	104.0	8.0
Parkview Lane	5.0	5.0	9.0	4.0	17.0	3.0
Source	10.0	7.5	-*	-	23.0	7.0
Garden Grove						
Sandra	12.5	2.5	9.0	7.0	9.0	3.0
Rockinghorse	15.0	5.0	64.0	3.0	33.0	7.0
Marshall Lane	30.0	7.5	12.0	6.0	9.0	4.0
Harris Street	10.0	7.5	3.0	2.0	65.0	9.0
Well 6064	7.5	5.0	4.0	3.0	-	-
Well 6321	-	-	12.0	4.0	-	-
Irvine						
Paseo Picasso	11.0	9.9	12.0	5.0	8.0	4.0
Via Messina	43.0	1.1	6.0	4.0	2.0	2.0
Arbor Lane	103.0	9.9	2.0	2.0	32.0	6.0
Parkview Lane	1.0	1.0	37.0	5.0	20.0	4.0
Source	-	-	19.0	4.0	-	-

(continued)

TABLE 30 (continued)

Location	WINTER frequency of fungal colonies	 fungal species	SPRING frequency of fungal colonies	 fungal species	SUMMER frequency of fungal colonies	 fungal species
Garden Grove						
Sandra	33.0	14.0	5.0	3.0	9.0	3.0
Rockinghorse	6.6	3.3	33.0	15.0	26.0	10.0
Marshall Lane	8.9	8.9	10.0	8.0	33.0	2.0
Harris Street	3.3	3.3	5.0	4.0	12.0	5.0
Well 6064	-	-	65.0	4.0	-	-
Well 6321	-	-	35.0	4.0	-	-
Mean (± S.D.)	44.6(±112)	7.3(±4.9)	17.7(±18.7)	4.7(±2.9)	25.1(±25.5)	4.9(±2.5)
IRVINE SYSTEM frequency of fungal colonies and species Mean (±S.D.)					37.8(±92.8)	5.8(±4.1)
GARDEN GROVE SYSTEM frequency of fungal colonies and species Mean (±S.D.)					20.0(±18.6)	5.5(±3.3)
TOTAL STUDY frequency of fungal colonies and species Mean (±S.D.)					28.9(±66.3)	5.6(±3.7)

*Not determined

TABLE 31. PEARSON'S r CORRELATIONS BETWEEN FREQUENCY OF FUNGAL COLONIES AND PHYSICAL PARAMETERS AND FUNGAL SPECIES (Level of Significance $p < 0.05$)

	Frequency of fungal species	Turbidity	pH
Irvine (n = 27)	.7	NS	.6
G. Grove (n = 29)	NS	NS	NS
Winter (n = 18)	.7	.9	.8
Spring (n = 21)	NS	NS	NS
Summer (n = 17)	.5	NS	NS
Total (n = 56)	.6	NS	.5

Fungal Species Correlations

In the Irvine system, of the physical parameters examined, only redox potential correlated significantly with the number of fungal species. Once again, none of the bacteriological parameters correlated significantly (Table 32).

In the Garden Grove system, none of the physical or bacteriological parameters correlated significantly with the number of fungal species.

Some differences were observed between winter, spring and summer correlations. The most apparent was the decreased correlation between numbers of fungi and turbidity from the winter sample ($r = 0.6$) to the spring sample ($r = 0.45$). In spring temperature correlated significantly ($r = 0.5$) whereas in summer as well as overall, none of the physical or bacteriological parameters showed correlations above ± 0.5.

Cluster Analysis and Discussion of Fungal Data

The cluster pattern utilizing mycological, physical and bacteriological parameters for the 8 sites is indicated in Figure 13. (Due to insufficient information, the 3 sources were not included in this analysis.) The clustering diagram indicates that the Irvine sites are more similar to each other than to the Garden Grove system. The Garden Grove sites, Sandra, Marshall and Rockinghorse, in turn are relatively similar to each other, whereas Garden Grove Harris is considerably different from all the other sites studied.

```
AMALG.
DISTANCE

                    1   2   3   4   7   6   5   8

                    *   *   *   *   *   *   *   *
 8.185              - + -   -   -   -   -   -   -
 8.644                - + - -   -   -   -   -   -
11.345                  - + - - -   -   -   -   -
                          -         -   -   -   -
                          -         - - + -     -
                          -   - + - - - -       -
                                - + - - - - - - -


Case 1 = Irvine            Paseo Picasso
     2 = Irvine            Via Messina
     3 = Irvine            Arbor Lane
     4 = Irvine            Parkview Lane
     5 = Garden Grove      Sandra
     6 = Garden Grove      Rockinghorse
     7 = Garden Grove      Marshall Lane
     8 = Garden Grove      Harris Street
```

Figure 13. Clustering Analysis of the Study Sites.

TABLE 32. PEARSON'S r CORRELATIONS BETWEN FREQUENCY OF FUNGAL SPECIES AND PHYSICAL AND FUNGAL COLONIES
(Level of Significance $p < .05$)

	Frequency of fungal colonies	Turbidity	Temperature	pH	Redox potential
Irvine (n = 27)	.7	NS	NS	NS	.5
G. Grove (n = 29)	NS	NS	NS	NS	NS
Winter (n = 18)	.7	.6	NS	.5	NS
Spring (n = 21)	NS	NS	.5	NS	NS
Summer (n = 17)	.5*	NS	NS	NS	NS
Total (n = 56)	.6	NS	NS	NS	NS

*Not significant to .05.

Comparison of the two distribution systems indicated that despite chlorination, the Irvine system exhibited a higher mean fungal count than the unchlorinated Garden Grove system. (As pointed out by Standard Methods, 14th edition, 1975, the chlorine resistance of fungal isolates is relatively unknown and these data would probably provide results concerning this question). The Irvine system fungal counts also showed a higher standard deviation, suggesting that this system experiences greater variation in fungal numbers. Further studies are required before the individual locations within the two systems can be statistically analysed.

It appears from these limited results that fungi exhibit some seasonal occurrence and that the factors influence this occurrence change over time. Continuation of the study for at least another season would have been desirable to allow a more definite evaluation. It appears at this stage that the fungal numbers exhibited a maximum in winter and a minimum in spring-summer.

The identification of fungal isolates to the genus level is highly desirable. Initial observations indicated that most isolates were members of the Deuteromycetes, particularly the Moniliales.

In the chlorinated system *Sporocybe* and *Penicillium* each comprised approximately 28% of the colonies isolated. *Acremonium* and *Paecilomyces* accounted for 10-15% of the colonies, whereas the other genera identified occurred with a mean frequency below 10 percent. In the unchlorinated system, *Penicillium* and *Acremonium* each comprised approximately 25% of the isolates, *Sporocybe* and *Paecilomyces* comprised about 15% and each of the other isolates indicated a mean frequency below 5 percent.

These four most frequently occurring genera were all members of the Deuteromycotina and are generally regarded as less important from the public health view than certain other fungi such as Candida and Aspergillus fumigatus. However, their presence could enhance colonization of distribution systems by providing: i) increased nutrients at the microhabitat level, and ii) initial colonization of surfaces, thus enhancing subsequent colonization by other microorganisms. Sporocybe and Acremonium are two leaf and wood decomposing imperfect fungi that have been previously recorded in some aquatic habitats. Paecilomyces is also a leaf and wood decomposing imperfect fungus with a somewhat wider aquatic distribution than the above two genera. Penicillium is also a leaf and wood decomposing imperfect fungus with a somewhat wider aquatic distribution than the above two genera. Penicillium is one of the most widely distributed fungal genera, containing leaf and wood decomposers as well as species with more specialized nutritional preferences and potential medical importance.

There have been no previous studies of fungal occurrence in drinking-water distribution systems, so the results of the present investigation cannot be compared to other studies. It seems reasonable that the fungi, a group of microorganisms well represented in other environments, would also be found in drinking-water distribution systems. The most important question from the environmental point of view is not so much their presence in such environments, as their physiological condition and activity. A combination of membrane filtration and vital staining (19, 85, 116) may provide answers to some of these questions.

Coliform Suppression

During this study a total of 2,033 bacteria were isolated and identified from the two water distribution systems investigated. Of these isolates, 1,144 were screened using the agar-overlay method for their ability to produce BLS specific for an E. coli, a Klebsiella sp. and an Enterobacter aerogenes strain. A total of 44.0% of the isolates were not examined because they were no longer viable at the time of BLS screening.

To determine if the die-off of isolates affected the reliability of the sample frequencies, mortality type, date, and site were computed (Table 33). There was a 36% die-off of isolates from the IRWD system and a 47% die-off of isolates from the Garden Grove system. From Table 33 it can be concluded that the die-off among all genera (except Enterobacter) was greater for the Garden Grove isolates than for the IRWD system isolates. The genera Pseudomonas, Acinetobacter and Klebsiella showed the greater differences, while the Flavobacterium/Moraxella group displayed similar mortality in both systems.

The date of bacterial isolation affected the isolate die-off rates in the IRWD and Garden Grove systems similarly (Table 33). The smallest losses were observed from the most recent samples (10/11/79, 8.4% in the IRWD system and 11.4% in the Garden Grove system). However, low die-off also occurred with the Irvine sample isolates in late January (12.9%). Die-off percentages by date (Table 33) ranged from 8.4% to 62.9% for the IRWD system samples and from 11.7% to 61.2% for the Garden Grove system samples. The date-related

TABLE 33. DIE-OFF CHARACTERISTICS (%) OF BACTERIA BY GENERA AND DATE OF ISOLATION DURING STORAGE AT 4°C

	Acinetobacter	Pseudomonas	Pasteurella	Pseudomonas/ Alcaligenes	Flavobacterium/ Moraxella	Klebsiella	Serratia	Enterobacter
Irvine	20.2	6.25	-	22.6	36.2	23.8	0.0	25.0
	(99)	(16)	-	(106)	(296)	(63)	(6)	(4)
Garden Grove	42.1	62.5	30.4	35.9	40.4	38.9	11.1	0.0
	(171)	(8)	(23)	(290)	(277)	(108)	(9)	(4)

	1/2/79	1/25/79	4/26/79	5/24/79	7/19179	7/26/79	10/11/79
Irvine	-	12.9	62.9	-	39.4	64.2	8.4
	-	(201)	(186)	-	(94)	(106)	(119)
Garden Grove	59.7	-	61.2	52.9	46.9	-	11.7
	(313)	-	(214)	(278)	(296)	-	(230)

( ) = sample size
- = no sample collected

die-offs mean that the observed frequencies of BLS producers were underestimates of actual frequencies.

Mortality by site figures (Table 34) indicated that site-specific die-off was considerable. Excluding Well 23/9 because of small sample size (n = 40), die-off percentages ranged from 28.1% at Rockinghorse to 64.6% at Well 6064.

The data in Table 35 indicate that a site-specific phenomenon occurred in the production of BLS by the isolates. At Paseo Picasso, 34.9% of the isolates produced BLS specific for *E. coli*, *Klebsiella* sp. or *Enterobacter aerogenes*. Of this 34.9%, a total of 85.0% were *Flavobacterium/Moraxella*. Less than 8.0% of the isolates from the other 5 sites examined were capable of producing substances inhibitory for the three coliforms.

The data in Table 35 also show that the percentage of BLS producers varied between sample times, with a low of zero in the July 26 sample to a maximum of 22.1% in the October 11 sample. A small percentage of the bacterial population was capable of producing an inhibitory substance in the majority of samples. BLS producer occurrence peaked in January, July and October.

Of the isolates from the chlorinated IRWD system samples, 22.1% were capable of inhibiting at least one of the three coliform bacteria tested, compared to 7.9% for the unchlorinated Garden Grove system. Combining the data from both systems, 13.4% of the isolates examined were capable of producing BLS specific for at least one of the three coliform test organisms.

Sample location, date of sample collection and the genus of the producer strain significantly influenced the degree and frequency of inhibition of the three coliforms (Tables 36 and 37). However, due to the relatively small "Cramers V" association coefficients, the relationships are significant, but not extremely strong. This statistical relationship implies that other factors not controlled for were more important in governing the occurrence of BLS-producing bacteria in the water distribution systems.

The size of the zone of inhibition of a lawn of *E. coli* varied according to the site from which the BLS-producing isolates were obtained ($F = 20.7$, $p < .001$). The date of isolation appeared to be of equal importance for both *E. coli* and *Klebsiella* sp. ($F = 7.5$, $p < .001$), but of lesser importance for *Enterobacter aerogenes* ($F = 2.1$, $p < .05$).

The number of bacteria capable of inhibiting the growth of the three test organisms is shown in Table 38. Of the isolates tested, it was found that 3.9% of the bacteria were capable of inhibiting *Enterobacter aerogenes*, 5.8% inhibited *Klebsiella* sp. and 7.6% inhibited *E. coli*. Of the *Enterobacter* species examined, approximately 28.0% produced BLS which were specific for *Enterobacter aerogenes*, *E. coli* and *Klebsiella* sp. As expected, coliform isolates from the distribution system generally exhibited strongest production of BLS (gauged by zone size) specific for the coliform test microorganisms (*Enterobacter aerogenes*, *E. coli* and *Klebsiella* sp.). The *Flavobacterium/Moraxella* group was an important non-coliform inhibitor for all three types of

TABLE 34. BREAKDOWN OF ISOLATES LOST (%) DURING STORAGE BY SITE OF ISOLATION

	Irvine		Garden Grove			
	Source	Paseo Picasso	Well 6064	Well 6321	Well 23/9	Rockinghorse
% of nonviable organisms	38.5	34.8	64.6	56.2	15.0	28.1
Total number of isolates	319	387	365	457	40	469
Total number of nonviable isolates	123	135	236	257	6	132

TABLE 35. EFFECT OF SAMPLE LOCATION AND SAMPLING TIME ON THE BACTERIAL POPULATION PRODUCING BACTERIOCIN-LIKE SUBSTANCES

Site of Isolation	No. Tested	Percent of Isolates producing coliform inhibitory substance	Percent of Isolates producing inhibitor specific for: E. coli		Klebsiella sp.		E. aerogenes	
			No.	%	No.	%	No.	%
Irvine Source	196	5.6	3	1.5	3	1.5	5	2.6
Paseo Picasso	252	34.9	55	21.8	40	15.9	25	9.9
Well 6064	129	4.6	4	3.1	3	2.3	0	0.0
Well 6321	196	14.3	15	7.5	12	6.0	8	4.0
Well 23/9	34	2.9	1	2.9	0	0.0	0	0.0
Rockinghorse	337	5.9	9	2.7	8	2.4	7	2.1
Date of Isolation								
1/2/79	126	8.7	4	3.2	4	3.2	6	4.8
1/25/79	175	14.8	15	8.6	8	4.6	7	4.0
4/26/79	148	8.5	6	3.9	7	4.6	6	3.9
5/24/79	131	4.6	3	2.3	2	1.5	1	0.8
7/19/79	214	13.6	15	7.0	13	6.1	6	2.8
7/26/79	38	0.0	0	0.0	0	0.0	0	0.0
10/11/79	312	22.1	44	14.1	32	10.3	19	6.1

TABLE 36. ANALYSIS OF VARIANCE (ANOVA): SIZE OF THE ZONE OF INHIBITION FOR E. COLI, KLEBSIELLA SP. AND E. AEROGENES BY SITE, DATE AND BACTERIAL GENERA

Inhibition zone diameter	Site	Date	Bacterial genus
E. coli	F = 20.7	F = 7.5	F = 5.9
	p = <.001	p = <.001	p = <.001
Klebsiella sp.	F = 6.7	F = 7.5	F = 5.0
	p = <.001	p = <.001	p = <.001
E. aerogenes	F = 8.6	F = 2.1	F = 4.6
	p = <.001	p = <.001	p = <.001

TABLE 37. CHI-SQUARE ANALYSIS: FREQUENCY OF COLIFORM-SPECIFIC BLS PRODUCERS BY SITE, DATE AND BACTERIAL GENUS

Coliform	Site	Date	Bacterial genus
E. coli	X^2 = 118.9	X^2 = 90.5	X^2 = 91.7
	p = <.001	p = <.001	p = <.001
	V = .24	V = .21	V = .21
Klebsiella sp.	X^2 = 79.8	X^2 = 55.4	X^2 = 69.3
	p = <.001	p = <.001	p = <.001
	V = .16	V = .16	V = .18
Enterobacter aerogenes	X^2 = 43.7	X^2 = 27.1	X^2 = 53.3
	p = <.001	p = <.001	p = <.001
	V = .15	V = .12	V = .16

TABLE 38. NUMERIC BREAKDOWN OF BACTERIAL GENERA PRODUCING BACTERIOCIN-LIKE SUBSTANCES FOR *ENTEROBACTER AEROGENES*, *KLEBSIELLA* SP. OR *ESCHERICHIA COLI*

Group	Total No. Tested	Inhibition of *E. aerogenes*		Total No. Tested	Inhibition of *Klebsiella* sp.		Total No. Tested	Inhibition of *E. coli*	
		No.	%		No.	%		No.	%
Actinomycetes	44	1	2.2	44	2	4.5	44	1	2.2
Acinetobacter sp.	178	0	0.0	178	8	4.5	178	8	4.5
Pseudomonas sp.	18	0	0.0	18	0	0.0	18	2	11.1
Pasteurella sp.	16	0	0.0	16	4	25.0	16	5	31.3
Pseudomonas sp./ *Alcaligenes* sp.	268	4	1.5	267	2	0.7	267	5	1.9
Flavobacterium sp./ *Moraxella* sp.	351	28	8.0	353	37	10.5	353	53	15.0
Klebsiella sp.	114	4	3.5	114	8	7.0	114	3	2.6
Serratia sp.	14	0	0.0	14	0	0.0	14	2	14.3
Enterobacter sp.	7	2	28.6	7	2	28.5	7	2	28.6
Unknown[1]	64	5	7.8	64	2	3.1	64	6	9.4
Unidentified[2]	70	1	1.4	70	1	1.4	70	0	0.0
Totals	1144	45(3.9%)		1145	66(5.8%)		1145	87(7.6%)	

[1] Unidentifiable by Lassen method.
[2] Not yet identified.

coliforms; 8.0% produced substances inhibitory for *Eneterobacter aerogenes*, 10.5% for *Klebsiella* sp. and 15.0% were capable of inhibiting *E. coli*.

Some representatives of all the genera tested were capable of inhibiting at least one of the three coliforms. Relatively high percentages of the *Serratia* (13.3%), *Flavobacterium/Moraxella* (8.4%) and *Pseudomonas* (8.3%) groups appeared to produce BLS for two of the three coliform group organisms. Members of eight different bacterial genera had the ability to inhibit two of the three coliform test organisms. Only four of the bacterial genera examined were active against all three coliforms. These included the *Flavobacterium/Moraxella* group (0.4%), *Klebsiella* (0.6%), *Enterobacter* (25.0%) and unidentified genera (0.5%).

Flavobacterium/Moraxella comprised 57.1% of the bacteria producing BLS determined by this method, while *Acinetobacter*, *Klebsiella* and *Pseudomonas/Alcaligenes* groups comprised 9.1%, 7,8% and 7,1%, respectively.

Composition of the MPN tube genera. The predominant coliform in both the false-negative and positive BGB tubes was *Enterobacter cloacae*. *Enterobacter cloacae* was present in such low numbers in the positive LTB tube to preclude its isolation at a dilution of 10^{-6} (Table 39). The only difference between the positive and false-negative MPN tubes was the presence of the *Flavobacterium/Moraxella* group in the latter. Although the screening of representative isolates from the MPN tubes failed to demonstrate any BLS activity against the predominant coliforms from the same tubes, members of the *Flavobacterium/Moraxella* group have been previously shown to be common coliform antagonists (4).

The single most important factor which could introduce sample bias in this study was the differential die-off of bacterial isolates from the IRWD and the Garden Grove systems. The experimental design of this study precluded the determination of whether the die-off of stored isolates was completely random with respect to the isolates ability to produce BLS. There is no evidence in the literature to suggest that the ability to produce BLS confers survival advantages or disadvantages *in vitro*. Therefore, it is likely that the loss of isolates during storage decreased the ranks of both BLS producers and non-producers randomly and independent of genus, site location, or date of isolation.

The inhibition zone size and the frequency of BLS production varied with the site of sample collection, the date of sample collection, and the generic status of the bacterial isolates. Thus, a variety of environmental factors, both physico/chemical and biological, might influence the occurrence and frequency of bacteria which synthesize coliform-specific BLS in water distribution systems. Such factors may explain the marked differences observed in the percentage of BLS producers in the two systems studied. Part of this difference was undoubtedly due to the greater frequency of the *Flavobacterium/Moraxella* group in the IRWD system.

Mitchell and Yankofsky (86) concluded that the bactericidal properties of natural seawater for *E. coli* depended upon the native marine bacterial flora. However, others have proposed that antibiotic production by marine microorgan-

TABLE 39. GENERIC MAKE-UP OF THE ISOLATES FROM POSITIVE AND FALSE-NEGATIVE LTB AND EGLB TUBES

	LTB+	BGB+	LTB+	BGB+
Enterobacter cloacae	20×10^6*	26×10^6	10^6	24×10^6
Aeromonas hydrophila	310×10^6	14×10^6	460×10^6	29×10^6
Klebsiella sp.	10^6	1×10^6	10^6	10^6
Serratia sp.	10^6	3×10^6	10^6	15×10^6
Flavobacterium/ Moraxella sp.	90×10^6	1×10^6	10^6	10^6
Total	420×10^6	45×10^6	460×10^6	68×10^6
# of colonies isolated off plate count agar for identification	42	45	46	68

*Number of bacteria in the MPN broth

isms was not a significant antagonist of coliform bacteria in natural seawater (20). Because of the comparatively low density of microorganisms in drinking water it is unlikely that antibiotics would play a significant role in the suppression of coliform bacteria by non-coliform microorganisms.

It is difficult to demonstrate experimentally that bacteria produce BLS under natural conditions and, at present, *in situ* synthesis has not been quantified. Studies supporting the single-hit hypothesis of bacteriocin function (i.e., one molecule of bacteriocin is sufficient to produce a death response as opposed to several molecules) (54) improve the possibility that low levels of bacteriocin production might conceivably cause coliform injury or death *in situ*. However, contact between a coliform cell and a coliform-specific BLS molecule must still occur. Assuming uniform distribution of bacteria in water distribution mains, then low concentrations of BLS in the system water would reduce the probability of such contact occurring. Our data indicate that bacterial distribution is clustered rather than uniform at pipe surfaces (see Scanning Electron Microscopy section).

Bacterial colonization of pipe surfaces in water distribution systems has been demonstrated (4, 106). Such colonization might create a situation where BLS inhibition is more plausible. These microcolonies could be important sites of production of coliform-specific BLS by non-coliform bacteria. Approximately 10% of the isolates in this study produced BLS specific for at least one of three coliforms tested. This suggests that the potential for bacteriocin induced suppression of coliforms in these systems is considerable. False-negative MPN tubes (i.e., growth without gas production) have been recorded in 15 instances over the 27-month study period representing 5.2% of the water samples collected. The cause of these false-negative results is unknown, though it is conceivable they may involve BLS. When large numbers of BLS producers are inoculated with coliform bacteria into MPN presumptive medium tubes, suppression of the coliform organisms might occur. Such inhibition might explain the decrease observed by Hutchison et al. (56) in numbers of *E. coli* cells which had been exposed to a non-coliform bacterial antagonist. The preliminary MPN experiments described herein failed to provide support for BLS-induced suppression of coliforms in MPN tubes and additional related experiments must be performed in order to clarify this point. However, it is clear from this research that coliform-specific bacteriocins have the potential to suppress coliform detection in MPN tests. Whether this is, in fact, a significant phenomenon is unknown at this time. What has previously been termed "bacterial antagonism" may be related to bacteriocins or bacteriocin-like substances produced by one bacterium and specific against another.

Scanning electron microscope evidence for bacterial colonization of a drinking water distribution system

Historically, the microbiology of potable water systems has been studied almost exclusively from a public health standpoint. A wealth of information and technology has accumulated over the years regarding the detection and enumeration of specific indicator and pathogenic bacteria and viruses in water distribution systems (43, 45, 100). However, in spite of this accumulation of knowledge, the incidence of waterborne disease in the United States continued to rise during the period 1971 to 1977 (29, 30). In part, this rise can be attributed to increased and better reporting of cases of waterborne illness to public health authorities, but also to a basic deficiency in our understanding of the specific environmental factors which regulate the occurrence and survival of diverse microorganisms (both pathogens and non-pathogens) in water distribution systems. Very little is known, for example, about the metabolic diversity and ecological status of many of the less familiar (yet far more predominant) apparently non-pathogenic microorganism which these systems harbor. Large numbers of a variety of different heterotrophic bacteria can be routinely isolated from both unchlorinated and chlorinated drinking water systems using standard microbiological techniques and media (43, 45). Many of these bacteria (e.g., Psuedomonas and Flavobacterium sp.) have been shown to be human secondary opportunistic pathogens (45). High in situ concentrations of such microorganisms might conceivably alter the system to produce a microenvironment more favorable for the survival and propagation of specific pathogenic bacteria. Alternately, they may be instrumental in inhibiting or suppressing specific pathogens that may gain access to the distribution system.

One aspect of the microbial ecology of drinking water which has received little attention thus far is the problem of bacterial attachment to solid surfaces and aquatic fouling. The ability of a particular bacterium to adhere to a surface vastly increases its access to potential food sources (2, 5), especially in very low nutrient environments (59). Therefore, the extent to which microbes become associated with the inner surfaces of pipes or with particulate matter suspended within the water column could significantly influence their survival and regrowth in distribution lines and reservoirs. In order to learn more about the nature and degree of association of resident microorganisms with solid interfaces in distribution systems, scanning electron microscopy was employed to examine the interior surfaces of excised pipe sections and also the surfaces of suspended particulate matter recovered from water samples.

A low magnification scanning electron micrograph of a representative area of the surface of the cement liner which coated the interior of the galvanized iron pipe section is shown in Figure 14. The actual surface of the cement liner was almost completely concealed beneath an amorphous mineral encrustation which appeared to be from 10 to 100 μm in thickness. This superficial mineralized layer contained a complex network of deep fissures and cavaties, effectively increasing the total available surface area of the pipe and providing a host of potential microhabitats for microorganisms to invade and colonize.

A topological SEM survey of this crustose layer at higher magnification revealed a number of morphologically distinct kinds of microcrystalline structures, probably inorganic in nature, either embedded within or protruding from the underlying amorphous layer of mineral deposits. The overall appearance of such microcrystalline structures ranged from compact aggregations of smooth spherical bodies, usually measuring a few micrometers in diameter (Figures 14-16), to more ornate, rosette-like arrangements (Figure 17), or clusters of flattened tentacle-like elements resembling blades of grass (Figure 18).

Because the chemistry of the pipe surface might strongly influence the ability of certain microorganisms to attach and multiply, the total elemental composition of the encrusting mineral layer was determined using x-ray energy dispersive microanalysis (x-ray EDX). The pipe surface shown in Figure 19a

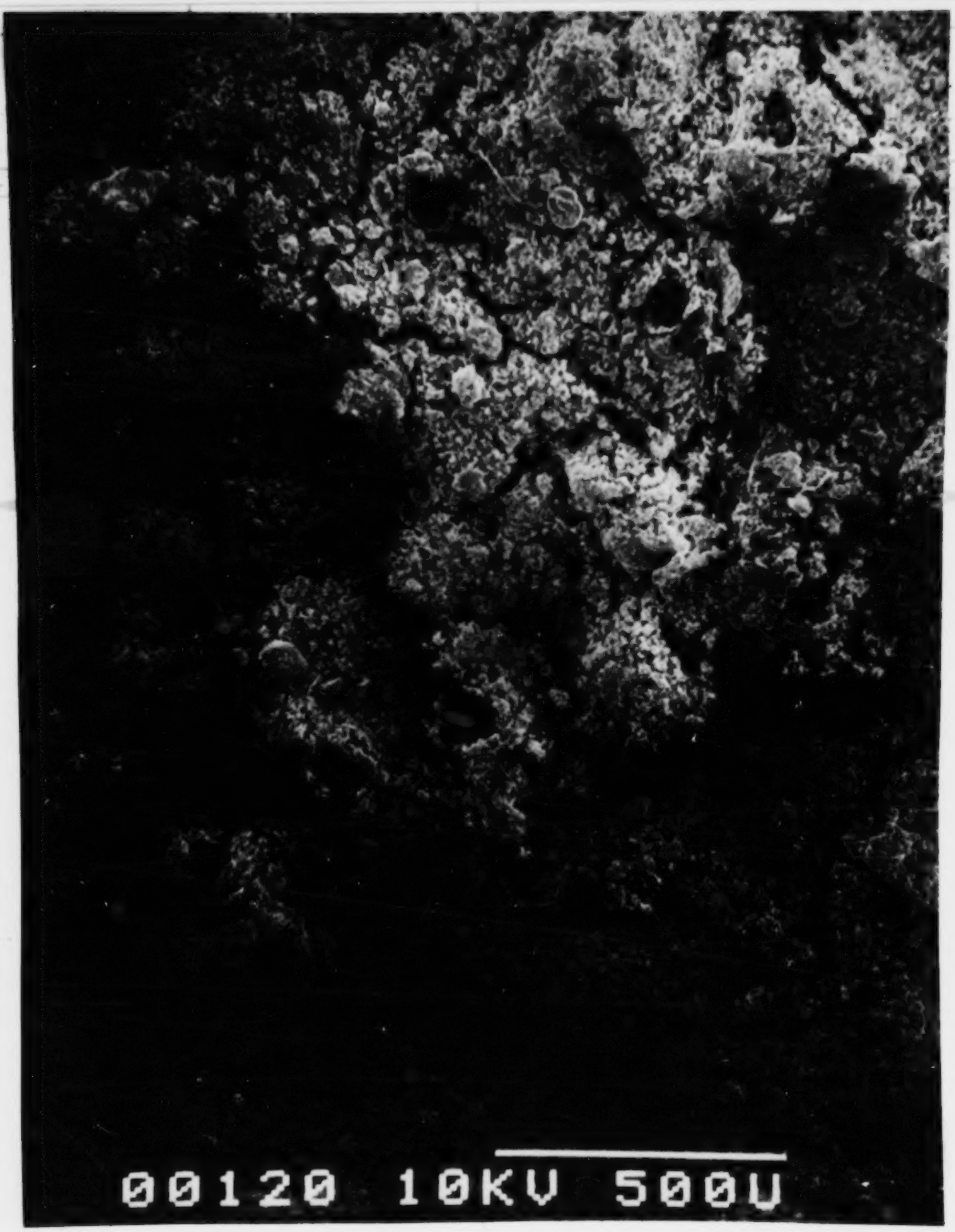
00120 10KV 500U

Figure 14. Scanning electron micrograph of cement liner of a galvanized iron pipe.

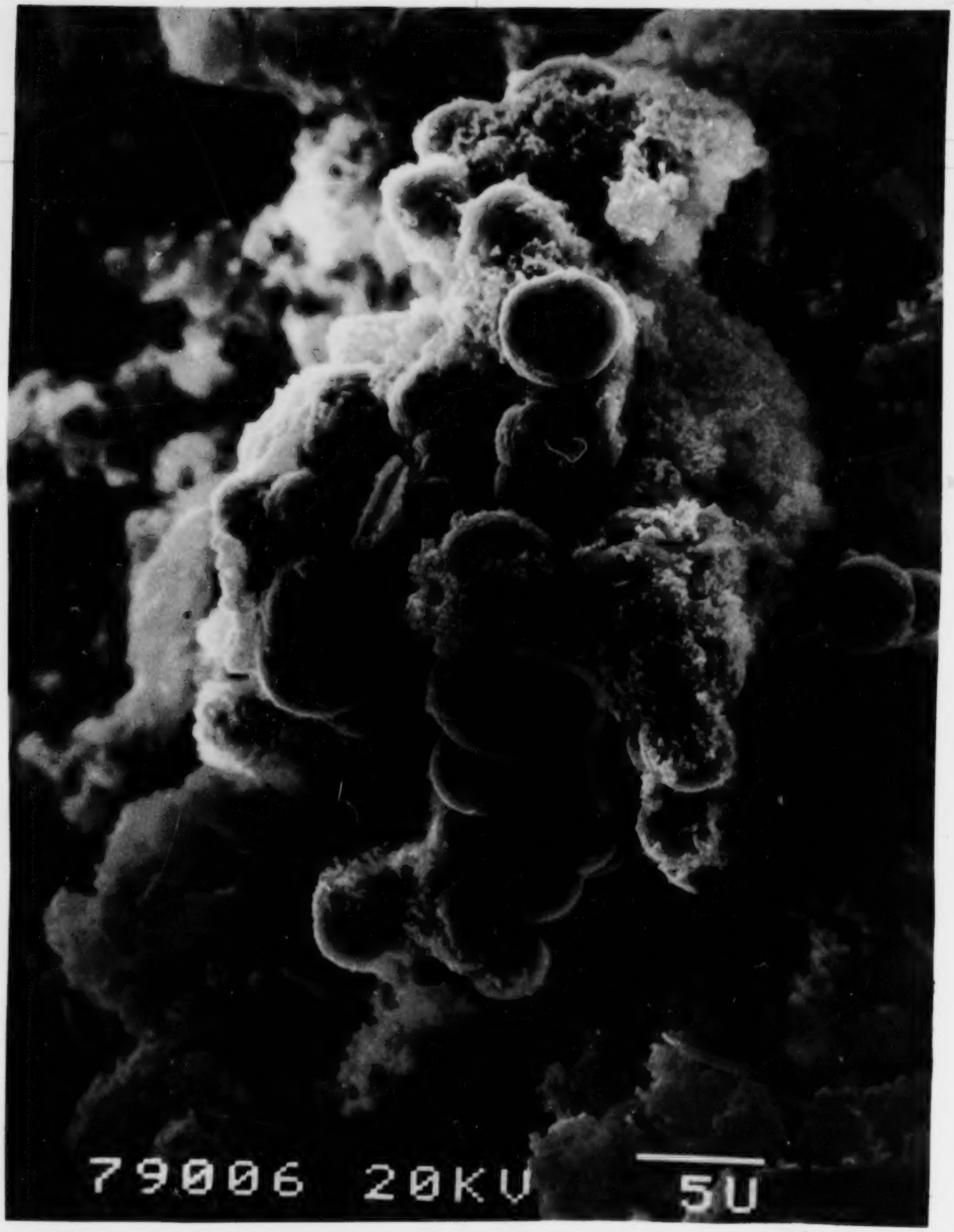
79006 20KV
5U

Figure 15. Inorganic deposits in galvanized iron pipe.

31536 10KU
5U

Figure 16. Inorganic deposits in galvanized iron pipe.

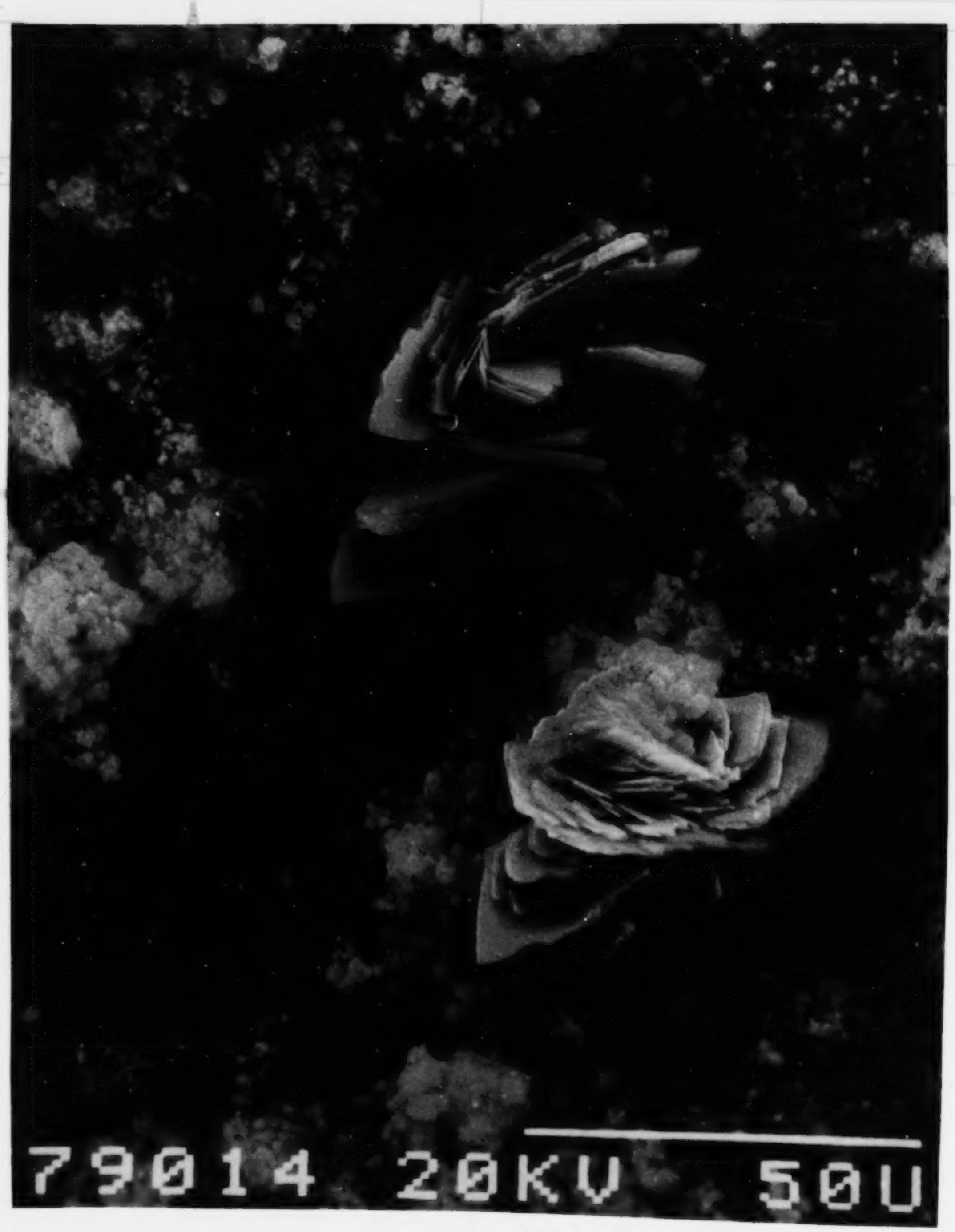
79014 20KV 50U

Figure 17. Inorganic deposits in galvanized iron pipe.

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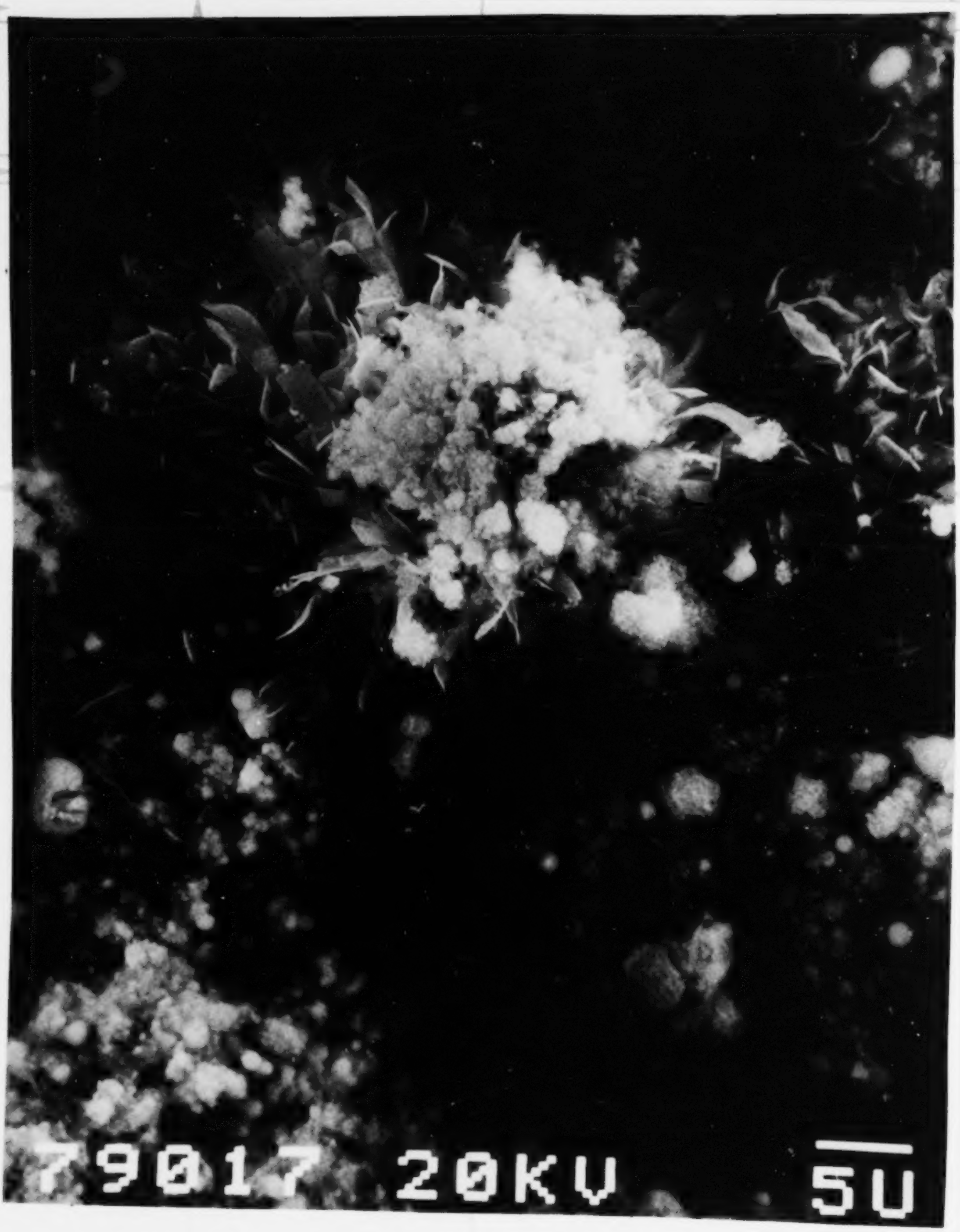

Figure 18. Inorganic deposits in galvanized iron pipe.

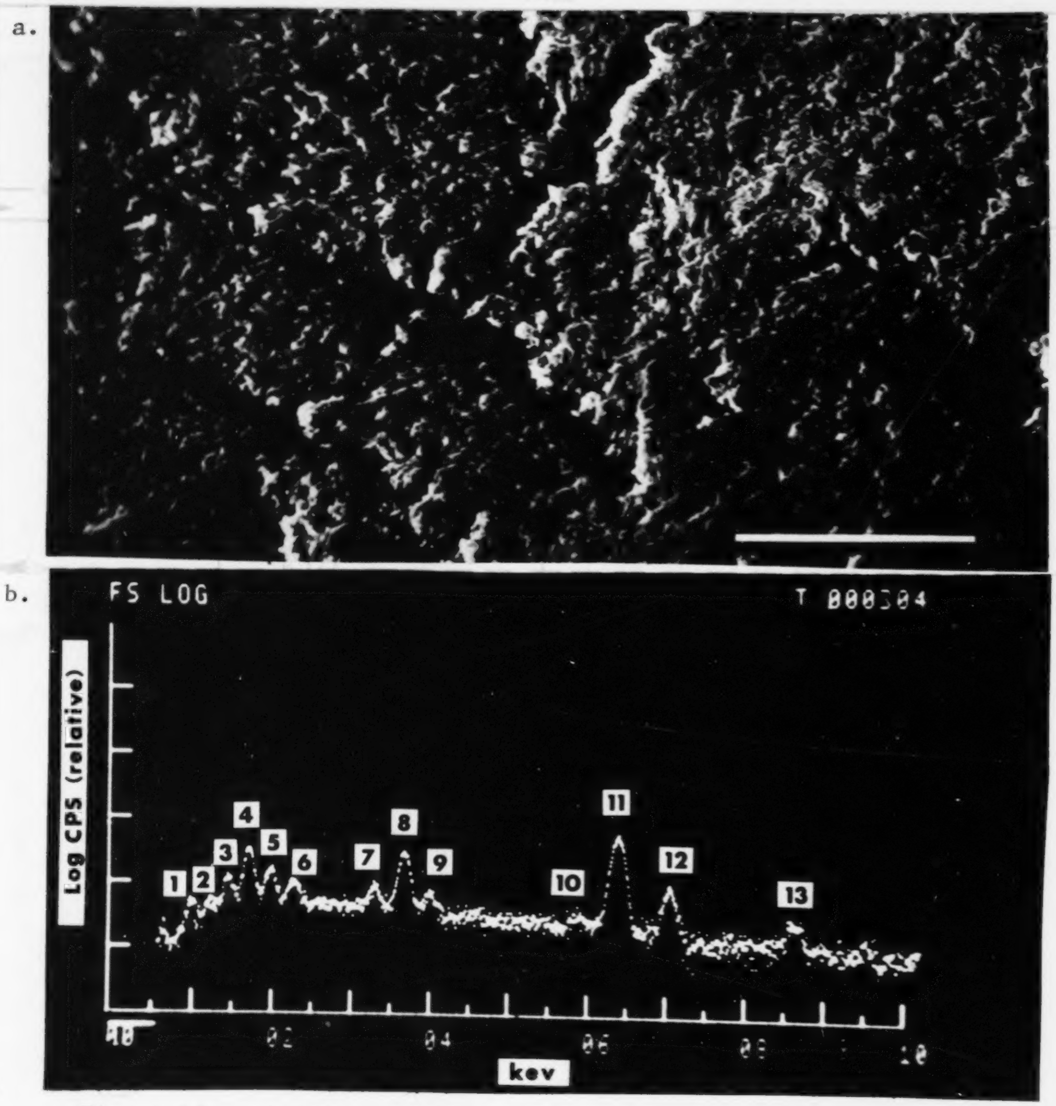

Figure 19. Scanning Electron Micrograph Energy Dispersive X-Ray Analysis of a Pipe Surface

a. Low-magnification scanning electron micrograph showing pitted and fractured texture of the encrusting mineral layer. Bar = 500 μm.

b. X-ray energy dispersive microanalysis of area of pipe surface shown in Figure 19a. Peak identities are as follows: (1) zinc L_α, 1.009 kev; (2) magnesium K_α, 1.25 kev; (3) aluminum K_α, 1.49 kev; (4) silicon K_α, 1.74 kev; (5) phosphorous K_α, 2.01 kev; (6) sulfur K_α, 2.31 kev; (7) potassium K_α, 3.31 kev; (8) calcium K_α, 3.69 kev; (9) calcium K_β, 4.01 kev; (10) manganese K_α, 5.89 kev; (11) iron K_α, 6.40 kev; (12) iron K_β, 7.06 kev; and (13) zinc K_α, 8.64 kev.

was subjected to x-ray EDM and the spectrum recorded from 0 to 10 kev is shown in Figure 19b. Thirteen x-ray energy maxima can be identified in the scan corresponding to a total of ten different elements. The individual energy maxima are (left to right in the scan pictured): zinc L_p peak (1.001 kev), magnesium K_p peak (1.25 kev), aluminum K_p peak (1.49 kev), silicon K_p peak (1.74 kev), phosphorous K_p peak (2.01 kev), sulfur K_p peak (2.31 kev), potassium K_p peak (3.31 kev), calcium K_p peak (3.69 kev), calcium K_β peak (4.01 kev), manganese K_p peak (5.21 kev), iron K_p peak (6.40 kev), iron K_β peak (7.06 kev), and zinc K_p peak (8.64 kev). Based on a visual comparison of peak heights, aluminum, silicon, phosphorous, sulfur, calcium, and iron appear to be the predominant elements detected in the pipe surface material. The proportion of each element comprising the mineral layer differed dramatically from that of the surrounding drinking water, where both iron and phosphorous were found to be relatively minor soluble constituents (.186 mg/l and .041 mg/l, respectively). Similarly, manganese and zinc which were present in very low concentrations in the water supply (.01 mg/l and .007 mg/l, respectively) were readily detected in the pipe surface material. A complete mineral analysis of the system water is provided in a separate report (107). Thus, it is probable that bacteria attached to the pipe surface are exposed to concentrations of minerals and/or organic micronutrients which are significantly different from those found in the water column itself. Such altered concentrations of minerals and nutrients could have a profound effect on the growth kinetics of attached microorganisms and their susceptibility to chemical disinfection processes.

An extensive survey of the microtopological features of the mineralized layer at high magnification in the scanning electron microscope revealed several areas where microcolonization by coccoid-shaped (Figures 20-22) or filamentous microorganisms (Figures 23-26) had apparently occurred. Such microcolonies were sparsely and randomly distributed along the pipe surface and were frequently associated with crevices in the mineral layer. The individual spherical bodies (bacteroids) which comprised some of the microcolonies closely resembled bacteria in size and morphology, generally ranging from about 0.5 to 1.0 μm in diameter. Occasionally, several of these spherical bacteria-like elements were observed linked together in chains (Figure 21), which appeared morphologically indistinguishable from commonly encountered bacteria, for example the gram-positive _Micrococci_ or chain-forming _Streptococci_. It is, of course, not possible to state with absolute certainty that all of the attached spherical elements observed in the scanning electron microscope represent actual microbiological entities. Final confirmation of the microbial nature of these structures will ultimately rest on their laboratory cultivation _in vitro_, or a demonstration of their _in situ_ metabolic activity. However, the likelihood that many of these structures are indeed bacteriological in nature is increased by the observation of the more complex chain-forming species mentioned above (Figure 21), filamentous forms (Figures 24 and 26) or specific extracellular structures which are typically found only in association with microorganisms. For example, the attachment of many bacteria to solid surfaces in aquatic habitats is frequently mediated by a network of extracellular mucopolysaccharide fibrils (i.e., glycocalyx) having a heteropolysaccharide of glycoprotein composition (38, 82, 114). Such extracellular fibrils can interact specifically with the substratum and have been frequently observed on the surfaces of bacteria living under conditions of

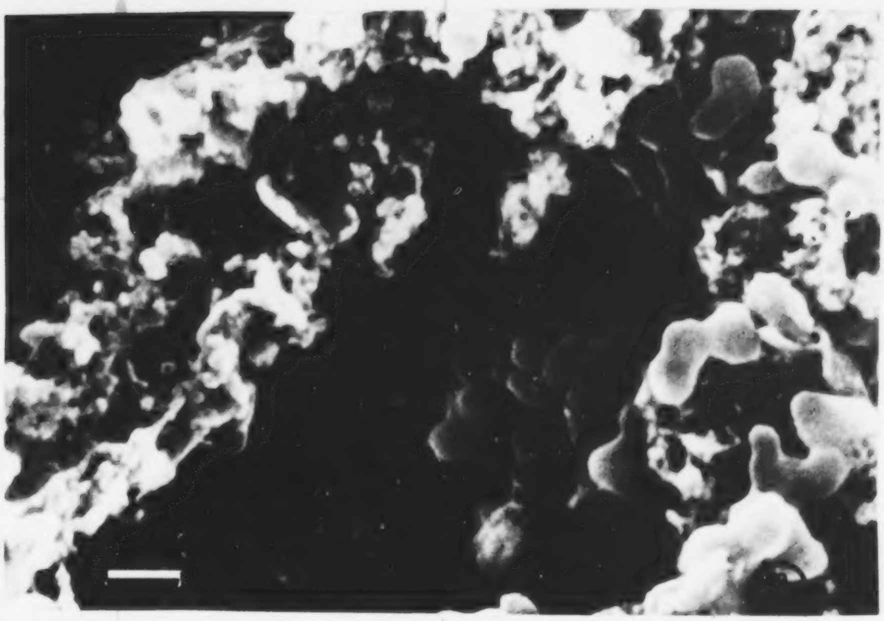

Figure 20. Microcolony of bacteria

Microcolony of coccoid-shpaed bacteria. Note smooth surface appearance of individual cells. Bar = 0.5 µm.

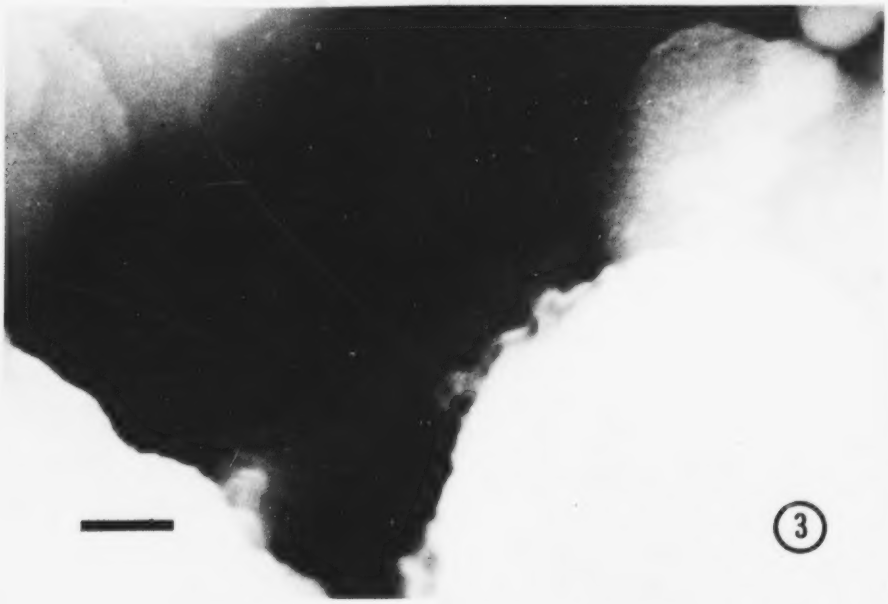

Figure 21. Chain of coccoid bacteria.

Linear chain of coccoid-shaped bacteria. Note rough surface texture of cells. Bar = .05 µm.

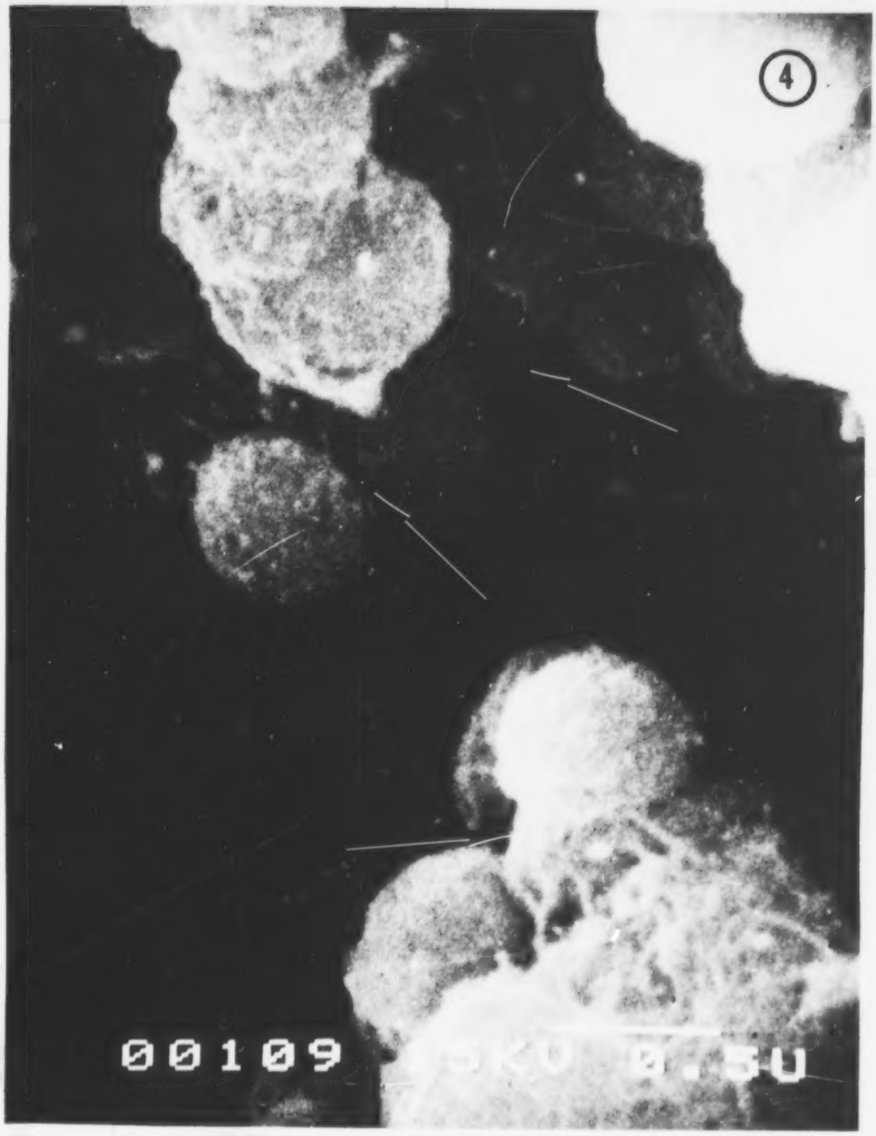

Figure 22. Surface morphology of bacterial microcolony. Attachment of bacteria to substratum by means of extracellular fibrillar material (arrows). Bar = 0.5 µm.

continuous hydrodynamic stress, such as in a fast flowing stream (42, 81). Extracellular fibrils, similar in appearance to those previously reported by other workers, were evident extruding from some of the coccoid-shaped bacteroids attached to the pipe surface (Figure 22). Formation of even a rudimentary glycocalyx would enable these presumptive bacterial cells to attach to relatively exposed areas of the pipe surface and still withstand moderate shear forces resulting from hydrodynamic turbulence within the system. Adhesion to surfaces would also provide the cell with increased nutrient availability, since a variety of trace mineral substances and organic compounds are known to be highly concentrated at the solid-liquid interface (81). Attachment would be particularly advantageous for slow-growing bacteria living under conditions of turbulent flow and oligotrophy which characterize municipal water supplies since adhesion to a surface would prevent such cells from washing out of the system prematurely.

Many of the attached coccoid-shaped cells exhibited a relatively smooth surface texture (Figure 20); others, however, were much rougher in appearance (Figure 22). Jannasch and Wirsen (59) have suggested that the rough surface texture of certain attached microorganisms residing near active sea-floor spreading centers might result from the depostion of inorganic metal oxide sheaths surrounding the bacteria. It is possible that a similar coating process might occur in ground water distribution systems from which various metallo-oxidizing bacteria have been isolated (31).

In addition to simple spherical-shaped bacteria, other cell types were observed which were morphologically identical to the prosthecate bacteria belonging to the genus Prosthecomicrobium (117). An example of one of these cells is shown in Figures 23 and 24. Such cells measured approximately 1.0 μm in diameter and were attached to the surface of the mineral layer by several thin (≃ 0.2 μm diameter) radiating appendages. As reported by Staley (117) in the original description of the genus Prosthecomicrobium, these filamentous appendates tapered distally to blunt ends. The appendages measured 1.0 to 2.0 μm in length and appeared to be evaginations of the cell wall rather than simple extruded fibers. Members of this genus are generally found in low nutrient or oligotrophic aquatic environments and show a marked tendency for surface adhesion. It may be possible for such microorganisms to become established on pipe surfaces in ground water distribution systems where oligotrophic conditions prevail.

Filamentous bacteria were the most frequently observed microorganisms (Figures 24, 25). They measured approximately 0.5 to 1.0 μm in diameter by 10 to 50 μm in length. The ends of many of these filaments were often hidden from view by mineral deposits, thereby preventing an accurate determination of their true filament length. They bore a striking morphological similarity to certain members of the Actinomycetales, namely the Streptomycetes, numerous strains of which have been frequently isolated (using standard plate count methodology) from water samples collected at hydrant sites proximal to the location where the pipe segment was recovered. Actinomycetes represent a routinely encountered taxonomic group of microorganisms in water distribution systems (12, 57) and have been linked to various taste and odor problems in drinking water supplies. The streptomycete-like filaments described here were generally attached to relatively exposed areas of the pipe surface and formed

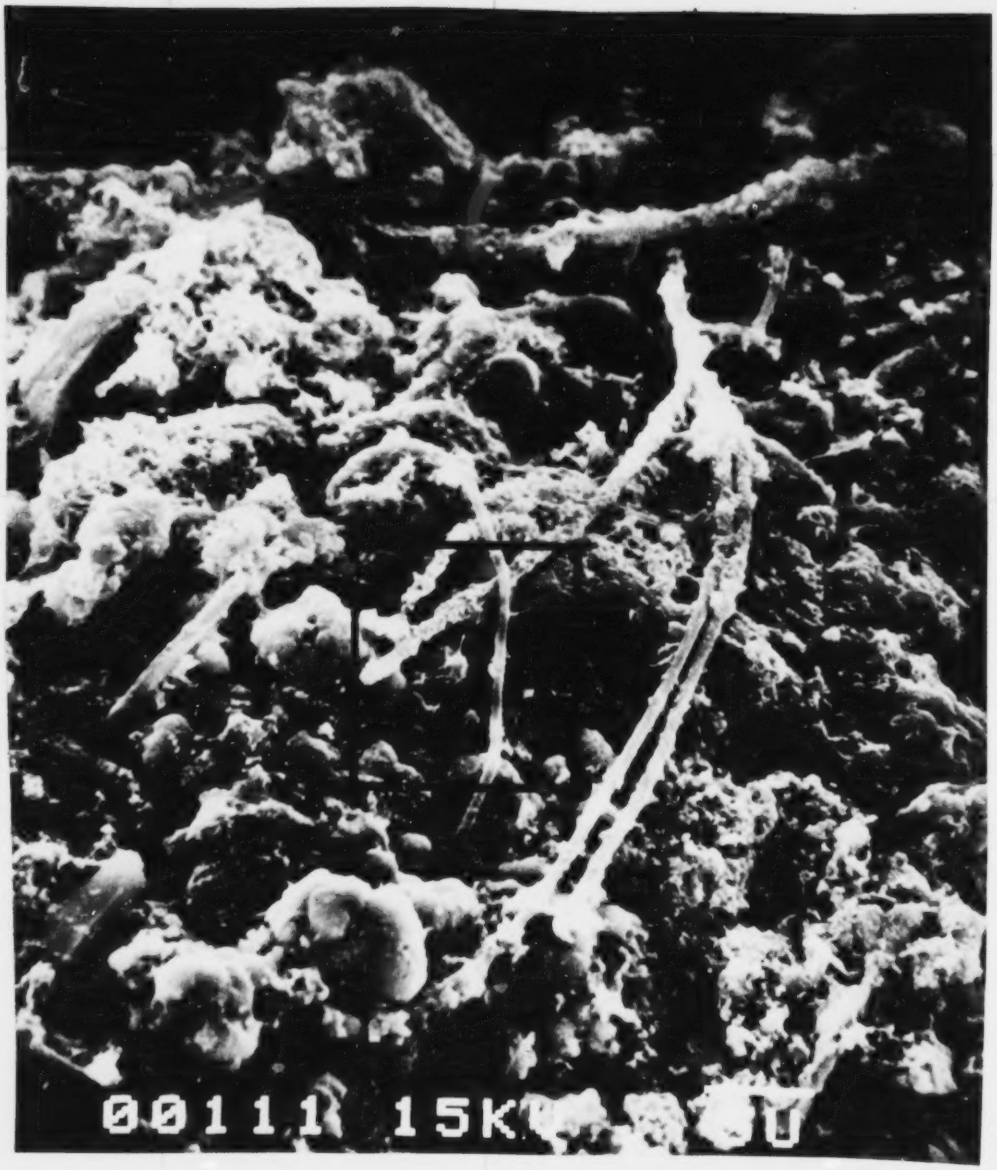

Figure 23. Prosthecomicrobium

Actinomycete-like filaments adhering to surface of mineral layer. Note coccoid-shaped cell (near center of delineated area) attached to filaments and mineral layer. Bar = 0.5 μm.

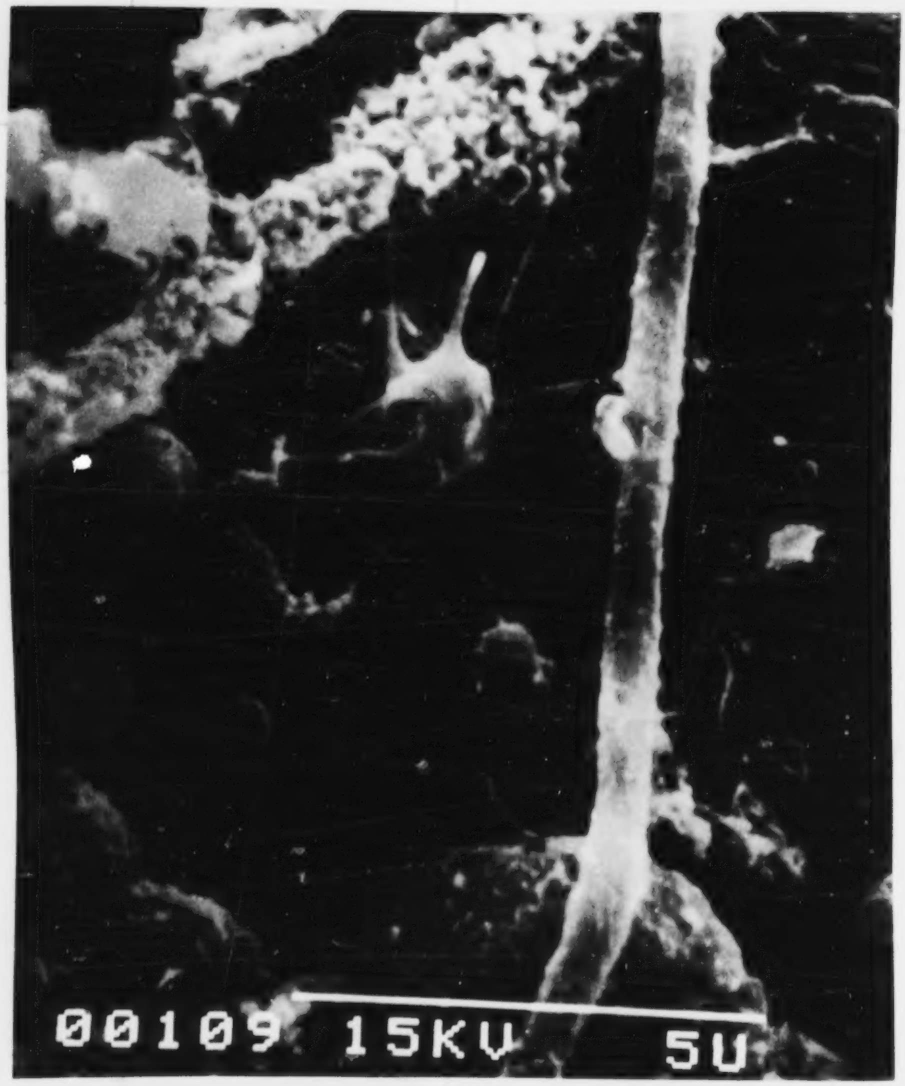

Figure 24. Prosthecomicrobium

Enlarged view, showing detail of suspected prosthecate bacterium belonging to the genus *Prosthecomicrobium*. Note layer of particulate debris covering actinomycete-like filament in upper portion of photograph. Bar = 5.0 µm.

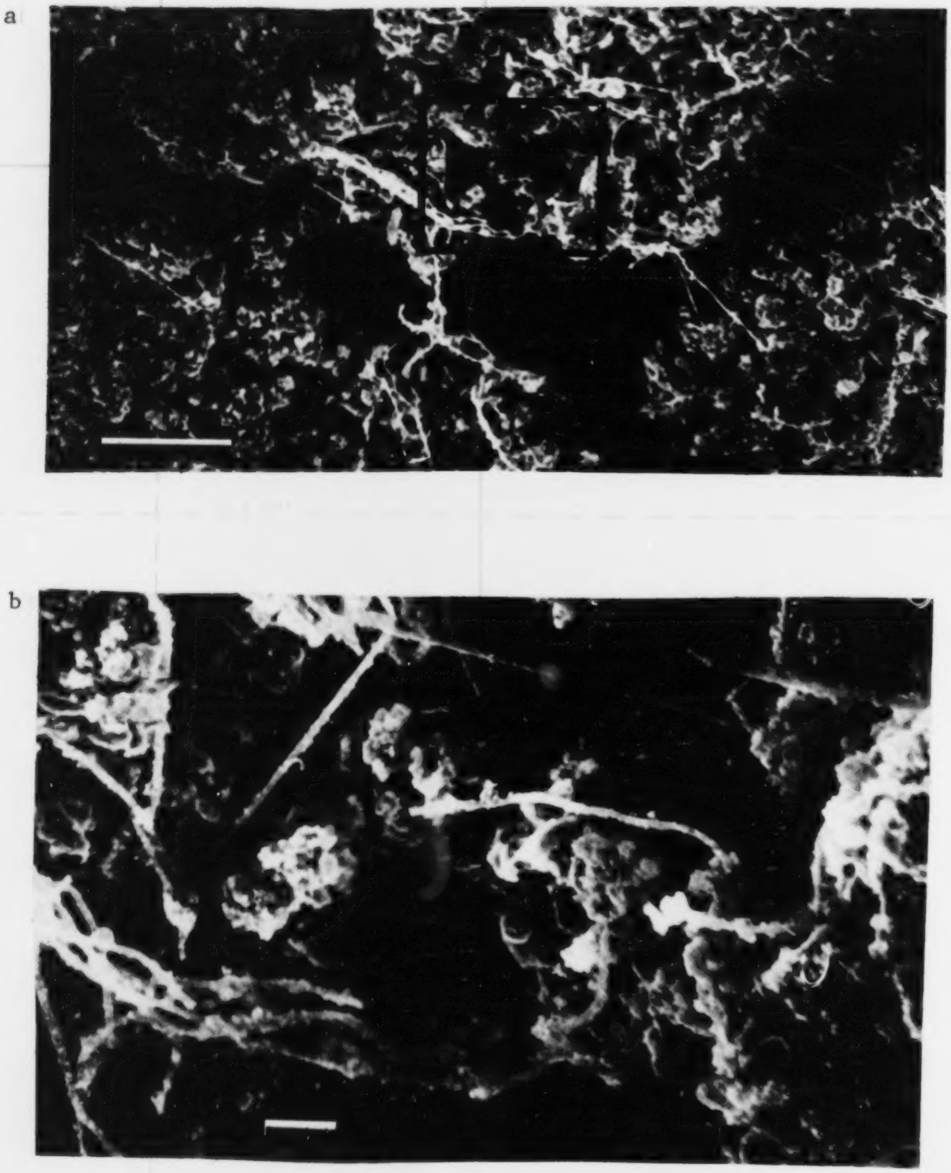

Figure 25. Filamentous Bacteria.

a-b. Region of microcolonization by filamentous actinomycete-like microbes. Bar = 50 μm (upper photograph). Close-up view of area delineated in square. illustrating trichomes penetrating into mineral layer. Some filaments appear coated with particulate debris. Bar = 5.0 μm.

loose networks of interwoven filaments. Many of the filaments penetrated into openings within the mineral layer, thereby appearing to firmly anchor the cells to the pipe surface. Whereas most of the trichomes had a relatively smooth surface texture (Figure 24), others were coated to varying degrees with a layer of particulate debris (Figures 24, 25). The individual particles comprising this layer measured approximately 0.1 to 0.2 μm in diameter and may have been held in place by an underlying sticky slime layer or glycocalyx, although this was not directly demonstrated in the photomicrographs.

In addition to the kinds of microorganisms described above, the autotrophic iron-oxidizing bacterium, Gallionella, was frequently observed in certain water samples and attached to pipe surfaces (Figures 26, 27). This microorganism was easily recognized by characteristic spiral-shaped stalks composed of numerous helically wound sub-microscopic protein fibrils (67). Gallionella obtains its energy chemolithotrophically presumably by the enzymatic oxidation of soluble ferrous iron to the ferric state (31, 68). This process results in the accumulation around the cell and stalk of insoluble ferric salts (principally the hydroxide form). The insoluble ferrous salts appear in the SEM photomicrographs as small spherical nodules adhering to the stalk surface. More detailed observations on the surface distribution, fine structure, and chemical composition of Gallionella cells recovered from this distribution system are described in a separate publication (107).

The SEM ultrastructural evidence reported here indicates that a large and diverse microbial community can adhere to and colonize the interior walls of underground drinking water distribution mains. In a similar recent study, Allen et al. (4) also reported extensive bacterial colonization of drinking water distribution pipes from other regions of the country. Such microbial colonization can apparently take place in spite of intermittent low-level chlorination of the system (0.1-0.2 mg free chlorine/l) during the summer months. It is possible that a large attached microflora could adversely affect the quality of drinking water supplies by metabolizing trace organic and inorganic constituents and excreting metabolic waste products. Microorganisms attached to pipe walls or present in partially anaerobic sediments in the bottom of the pipe in the peripheral regions of the distribution system are believed to be directly responsible for taste, odor and color problems in potable water systems (31, 57).

Proliferation of bacteria in microcolonies on pipe surfaces could also lead to a significant underestimation of the total number of viable microorganisms associated with a given water supply, thereby making accurate public health assessments of the water supply difficult or impossible to achieve. Further, extensive aggregation of cells or microcolonies on the surface of pipes in conjunction with the estrusion of extracellular slime layers, have the potential to dramatically reduce the effectiveness of conventional modes of disinfection, such as chlorination, and to predispose the pipe surface to further microbial fouling and corrosion processes (29, 57).

Iron bacteria in drinking water distribution systems: elemental analysis of Gallionella stalks using x-ray energy dispersive microanalysis

Fossilized "iron" bacteria belonging to the genus Gallionella were first

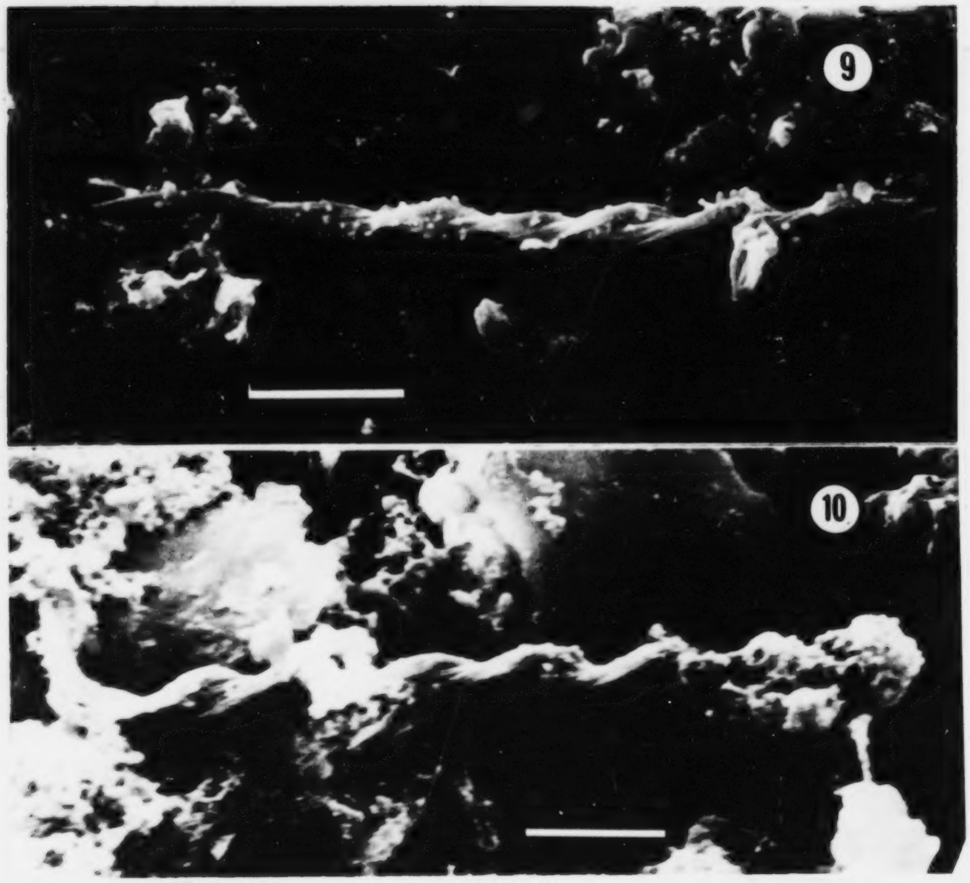

Figure 26. Gallionella Associated with Iron Pipe.

Gallionella stalk on surface of Nucelpore membrane filter. Note striated appearance of helical stalk. Bar = 5.0 μm (upper photograph. Gallionella stalk attached to pipe surface. Bar = 5.0 μm (lower photograph).

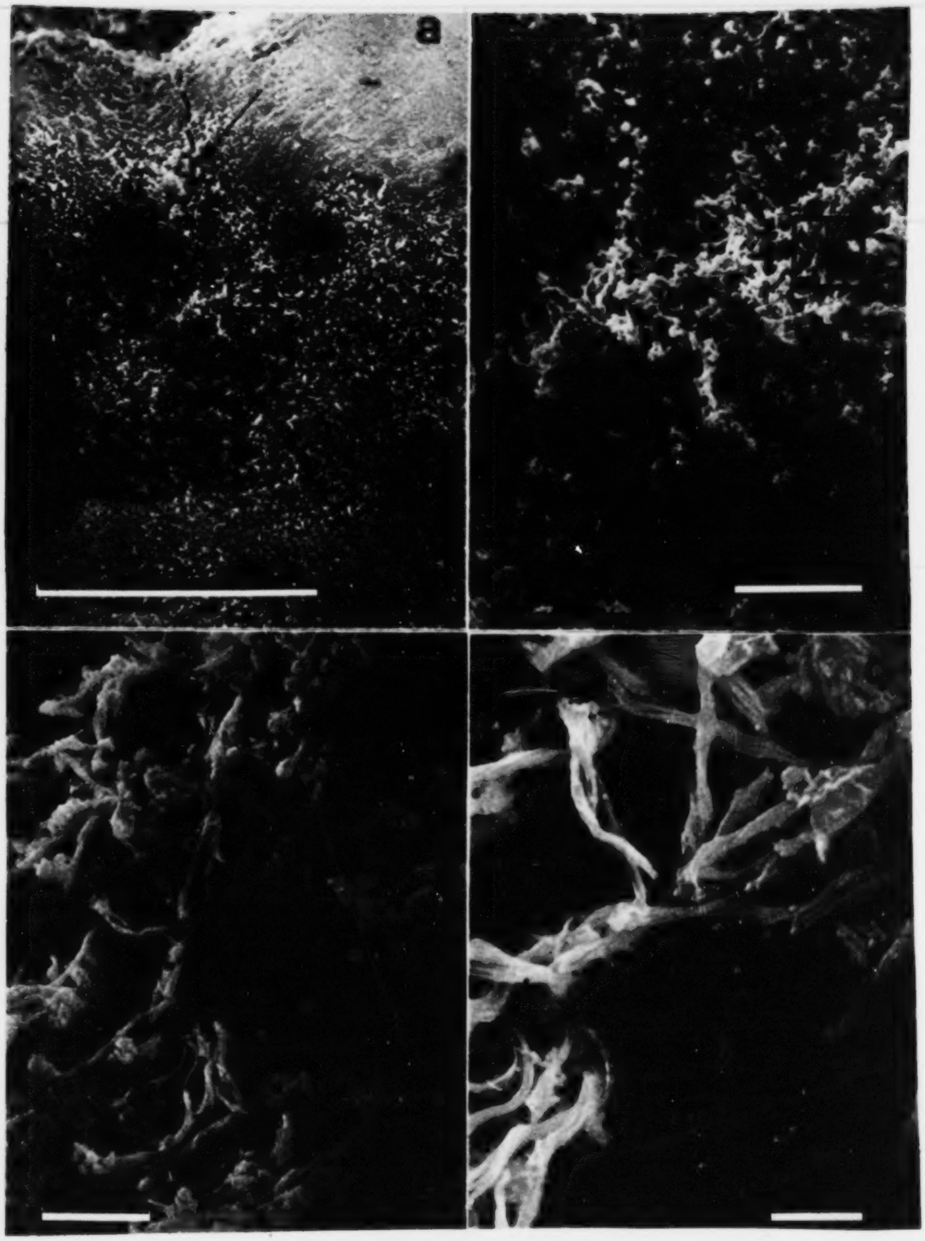

Figure 27. Gallionella Attached to Pipe Surface.

View of surface of rust-colored nodule from galvanized iron pipe showing flocculent type precipitate and Gallionella microcolonies (arrows in a); (b-d), increasingly higher magnification views of microcolony delineated by rectangle. Note typical striated helices of individual Gallionella stalks in d. Bars = 500 μm (a); 50 μm (b); 10 μm (c); 5.0 μm (d).

discovered in ochre mineral deposits by Ehrenberg in 1836 (34). It was Cholodny in 1924 (22), and later Perfil'ev in 1926 (98), however, who provided the first detailed descriptions of the morphology and developmental cycle of the genus. A number of workers subsequently studied the physiology and ultrastructure of this extraordinary microorganism and several recent literature reviews on these subjects have been published (31, 48, 53, 66, 78, 126). Gallionella has been found in a variety of soil and aquatic habitats, always associated with iron (48). The organism consists of a kidney-shaped mycoplasmidial cell body, deficient in the usual peptidoglycan component which imparts rigidity to the bacterial cell wall (7). For this reason Balashova (7) has suggested evolutionary kinship to the Mycoplasma, and to Metallogenium, a related, wall-less polymorphic iron bacterium. A single elongated stalk constructed from numerous helically wound, uniquely mineralized fibrils extends outward from the convex side of the cell of Gallionella. The protein nature of the stalk fibrils was demonstrated by Balashova (6) who used proteolytic enzymes to actively digest them. Some workers believe that the stalk itself may constitute the principle living reproductive element of this microorganism, and that the apical cell body develops only during transition to a motile stage in the life cycle (68). Hanert (51), however, contends that any Gallionella stalk growing slowly under natural conditions terminates in an apical cell. Though the exact function of the stalk remains obscure, it is sufficiently characteristic in appearance to permit microscopic identification of Gallionella in crude preparations.

In order to learn more about the nature and composition of the mineral encrustations on the surface of Gallionella cells, and their possible involvement in microbial fouling and corrosion processes, the microzonal distribution of this bacterium on pipe surfaces was examined in the scanning electron microscope. In addition, the elemental composition of the mineral precipitates coating the surfaces of Gallionella stalks obtained directly from the distribution system was determined by EDX.

When the surfaces of the rust-colored nodules from galvanized-iron pipes were examined using the SEM, they appeared to be coated with a superficial layer of flocculent precipitate. Inspection of the precipitate at higher magnification revealed dense microcolonies of Gallionella. Such microcolonies were randomly distributed over the nodule surface, and in some areas the entire nodule surface was covered with a mat-like layer of cells up to several µm in thickness (Figure 27a-d). Gallionella cell bodies were not observed, possibly having been obscured beneath the mineral deposits. Only the helical stalks, which tended to form the intertwined masses, were clearly discernible. Neither the macroscopic rust-colored nodules, nor Gallionella microcolonies were detected on connecting copper and asbestos-concrete pipes removed from a different but proximal location within the distribution system. Although an exhaustive search of the system was not made, Gallionella cells and microcolonies appeared to be predominantly associated with galvanized-iron pipes which were in various stages of deterioration due to corrosion processes.

Individual Gallionella stalks were found throughout the year in varying concentrations in water samples obtained from different wells or hydrant sites within the system. The relative abundance of Gallionella stalks and the corresponding physico-chemical characteristics of the water at selected well and hydrant sites are shown in Table 40. No stalks were detected in water samples obtained from well/reservoirs #6321 or 23/9, or well site #6064, which collectively supply more than 90% of the water for the three hydrant locations shown in Table 40. The stalks were observed in greatest numbers in older, dead-end pipe sections where the water flow rate was reduced to a minimum and lower *in situ* oxygen tensions and redox potentials prevailed (e.g., Marshall Lane). It is probably also significant that these bacteria were most numerous in galvanized-iron pipes where higher concentrations of soluble reduced iron species might be expected to occur. Similarly, McMillan and Stout (78) found *Gallionella* stalks primarily associated with rusty pipes and dead-end mains in the Chicago water distribution system. This is in conceptual agreement with the findings of Hanert (50) who reported that growth of *G. ferruginea* in laboratory culture was enhanced by low oxygen concentrations (0.1-0.2 mg O_2/l), whereas higher oxygen concentrations (> 2.75 mg O_2/l) were inhibitory.

The above data suggest that physico-chemical conditions prevailing at specific locations (or microhabitats) within the distribution system influence the survival and growth of *Gallionella* cells. The overall mineral composition of the distribution source water, which showed little variation over time, may be less influential in this regard. The concentrations of various ions in water from the three major source wells and from two of the hydrant sites in the distribution system are shown in Table 41. The minimum concentrations of iron reported in the literature that support the growth of *Gallionella* (and *Leptothrix*) in fast-flowing, well-aerated water, such as a pumping well, vary from about 0.2 to 0.5 mg/l (53). In this study, the concentration of iron in the source water was, on the average, about one order of magnitude less than these values (i.e., approximately 0.02 mg iron/l) and may therefore explain the absence of *Gallionella* from the well sites. Interestingly, the concentration of most ions, including iron, increased from the source water to the hydrant sites. As much as a 33-fold increase was observed in some instances in the total iron concentration from the source to the site. Iron concentrations at the hydrant sites were within the limits reported to support the growth of iron bacteria and may have been even higher where galvanized-iron pipes were installed, especially in dead-end sections. Other substances required for the autotrophic growth of *Gallionella*, such as dissolved oxygen, carbon dioxide/bicarbonate, ammonia nitrogen, phosphate, etc., were generally present in excess of growth limiting concentrations.

Examples of some of the waterborne *Gallionella* stalks obtained from the system are shown in Figure 28. These stalks were morphologically indistinguishable from those on pipe surfaces and consisted of at least 20 distinct helically coiled microfibrils (Figure 28). Each fibril measured approximately 0.04 to 0.05 μm in diameter and apparently extended the length of the stalk (Figure 28). Contrary to the findings of Hanert (50), no clear indications of transverse fission or segmentation of the fibrils into "microcellular reproduction units" were observed in the scanning electron microscope, although this may have been due to limitation in instrument resolution (about 10 nm). Intact stalks measured approximately 2 μm in diameter, though the overall lengths of the stalks exhibited considerable variation. Such variation in stalk length could have resulted from different length filaments being shorn from the pipe surface by increased hydrodynamic stress during sampling.

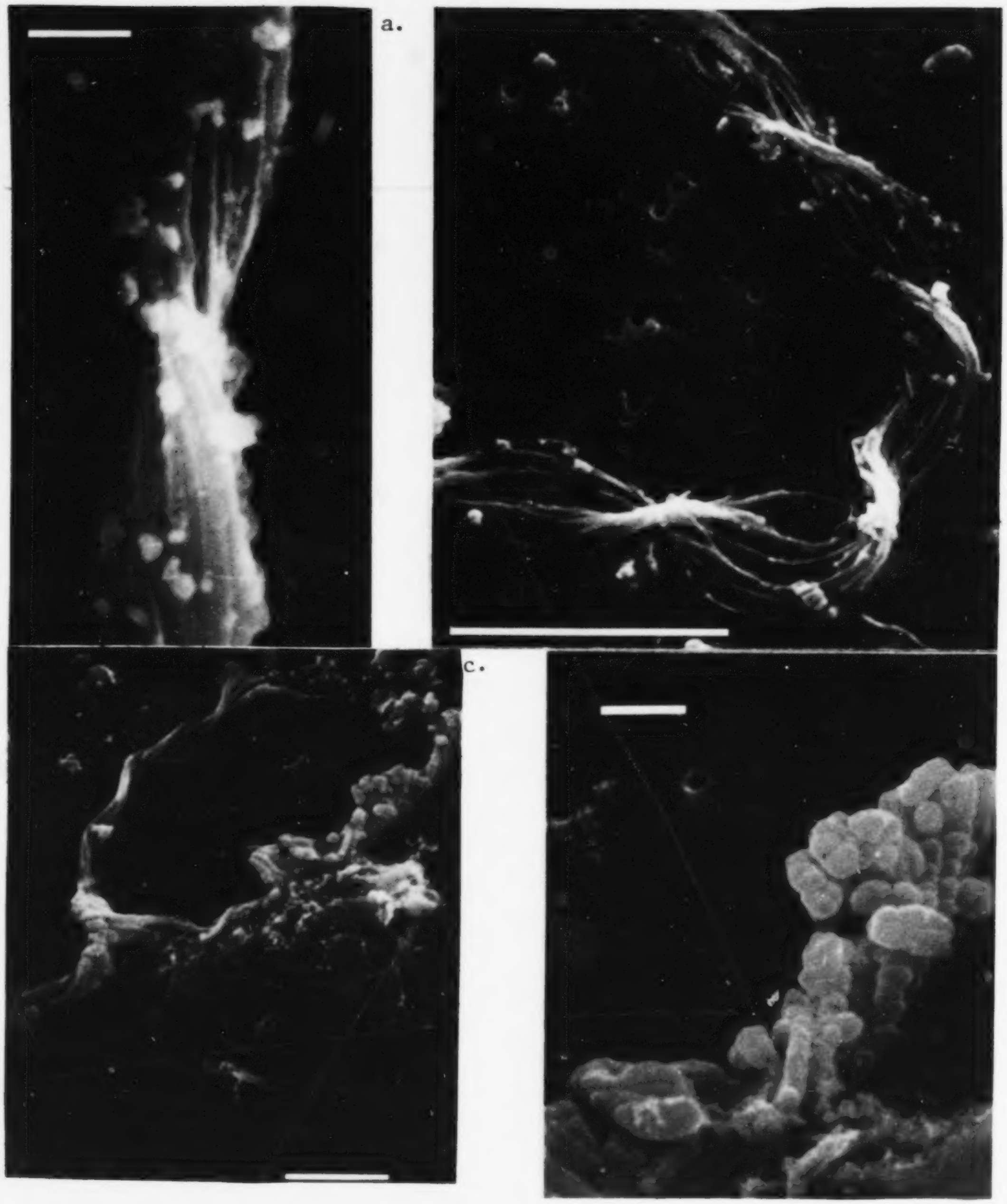

Figure 28. *Gallionella* from Distribution Water.

Selected views of *Gallionella* stalks recovered in water samples from the Marshall Lane site. Note individual microfibrils which compose stalks (*b*). Bars = 0.5 m (*a*); 5.0 μm (*b*); 10 μm (*c*); 5.0 μm (*d*).

TABLE 40. OCCURRENCE OF GALLIONELLA STALKS AND PHYSICO-CHEMICAL PROPERTIES AT SAMPLING SITES[1]

	Well Reservoir Sites			Hydrant Sites		
	6321	23/9	6064	Rockinghorse	Harris	Marshall
Gallionella[2] detected	ND	ND	ND	+	+	++++
	$\overline{X}$ ± S.D.	$\overline{X}$ ± S.D.	$\overline{X}$ ± S.D.	$\overline{X}$ ± S.D.	$\overline{X}$ ± S.D.	$\overline{X}$ ± S.D.
pH	7.28±.579	6.83±.528	7.46±.430	7.38±.414	7.41±.462	.46±.859
Temperature (°C)	19.3±1.36	20.5±2.68	18.3±.985	20.5±2.67	19.6±2.12	19.9±2.38
Turbidity (NTU)	.246±.105	.300±.127	.223±.093	5.35±22.9	8.96±31.5	8.78±15.4
Dissolved O_2 (mg/l)	7.57±1.12	7.93±.731	7.59±.698	7.20±1.05	7.23±1.03	7.12±1.14
Redox Potential (mv)	114±42.1	170±43.4	133±48.2	102±71.9	87.3±108	60.4±118
Pipe Material[3]	C	S	S	AC	AC	GI
Maximum Flow (gal/min)	-	-	-	4000	4000	60
Date Installed	1969/71	1966	1954	1955	1955	@1940
Dead End Section	no	no	no	yes	yes	yes

[1] Except for turbidity, parameters measured in situ bimonthly from 12/19/78 through 11/8/79. Data for Well 23/9 and Harris site collected 8/79 through 11.8.79. Water turbidity was measured (in standard Nephelometric units, NU) in a HIAC N-60 turbidimeter (1).

[2] ND = no Gallionella stalks detected in scanning electron microscope after filtering 20 ml of sample through a 3.7 cm Nuclepore membrane filter (0.2 μm pore size).

++++ = many stalks frequently observed.

[3] AC = asbestos-concrete pipe; GI = galvanized-iron pipe; C = concrete; S = steel.

TABLE 41. CHEMICAL ANALYSIS OF WATER FROM WELL AND HYDRANT SITES[1]

Component	Method of Analysis[2]	Well/Reservoir Sites[3]			Hydrant Sites[4]			Hydrant/Well Ratio
		$\overline{x}$	±	S.D.	$\overline{x}$	±	S.D.	
sodium	flame photometry	40.1	±	7.43	57.2	±	6.41	1.4
potassium	flame photometry	3.82	±	.482	4.41	±	.330	1.2
calcium	EDTA titration	80.8	±	9.84	89.4	±	19.8	1.1
mangnesium	EDTA (by calculation)	16.0	±	2.62	23.3	±	10.3	1.5
manganese	atomic absorption	.004	±	.005	.010	±	.010	2.4
total iron	atomic absorption	.023	±	.035	.186	±	.336	8.3
silica	colorimetric method	20.7	±	.212	21.9	±	.919	1.1
cobalt	atomic absorption	.002	±	.001	.002	±	.001	1.1
copper	atomic absorption	.035	±	.044	.008	±	.006	0.2
zinc	atomic absorption	.036	±	.055	.007	±	.007	0.2
bicarbonate	potentiometric titration	216.0	±	11.4	216.0	±	28.6	1.0
carbonate	potentiometric titration	.780	±	.425	.639	±	.312	0.8
carbon dioxide	calculated from alkalinity	8.77	±	8.94	9.48	±	10.2	1.1
chloride	mecuric nitrate	62.7	±	82.2	68.1	±	5.12	1.1
sulfate	turbidimetric	91.5	±	19.7	146.0	±	27.3	1.1
nitrate	brucine colorimetric method	17.0	±	14.1	18.4	±	8.2	1.1
ortho-phosphate	ascorbate method	.040	±	.032	.041	±	.049	1.0

(continued)

TABLE 41 (continued)

Component	Method of Analysis[2]	Well/Reservoir Sites[3]	Hydrant Sites[4]	Hydrant/Well Ratio
		$\overline{x}$ ± S.D.	$\overline{x}$ ± S.D.	
Kjeldahl-N	Kjeldahl method	.005 ± .001	.041 ± .049	15.0
amonia-N	phenate method	.022 ± .018	.020 ± .013	0.9

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[1] Quantities shown are in mg/l. Data accumulated bimonthly 12/19/78 through 11/8/79.

[2] For general description of analytical methods, see ref. (1).

[3] Arithmetic means for well sites 6321, 23/9, and 6064. Data included for well 23/9 was collected from 8/79 through 11/8/79.

[4] Arithmetic means for hydrant sites Rockinghorse and Harris. Due to incomplete data, the Marshall site was not included in this analysis. However, the Harris site is proximal to Marshall and therefore probably closely resembles it. Data included for the Harris site was collected from 8/79 through 11/8/79.

Some stalks were coated to varying degrees with small (0.2-0.5 μm) bud-like or nodular structures, possibly corresponding to the "spherical bodies" described earlier by Balashova (6) in ultra-thin sections of axenically-grown cultures of *G. filamenta* (Figure 29). Most of the stalks, however, were clearly encrusted with relatively large quantities of oxidized iron precipitates (Figure 28).

It was not feasible to perform x-ray energy dispersive microanalysis on *Gallionella* cells attached to the pipe surface due to the complex microtopography of the sample, which could have introduced significant electron scattering artifacts. Therefore, x-ray elemental analyses were performed on selected *Gallionella* stalks isolated directly from water samples. Figure 29b shows a continuous x-ray energy spectrum from 0 to 10 kev of the indicated portion of the filament shown in Figure 29a. Four major peaks were observed corresponding to the K_α energy lines of aluminum (K_α = 1.49 kev), silicon (K_α = 1.74 kev), calcium (K_α = 3.69 kev), and iron (K_α = 6.40 kev). Manganese (K_α = 5.21 kev), though present in measurable quantities in the water supply (Table 41), was not detected in the stalks, thus confirming the apparent inability of this microbe to oxidize this element. Smaller peaks were identified as the L_α line of zinc (1.00 kev), the K_α lines of phosphorous (K_α = 2.01 kev), sulfur (K_α = 2.30 kev), chlorine (K_α = 2.62 kev), copper (K_α = 8.04 kev), and zinc (K_α = 8.64 kev), and the K_β peak of iron (7.06 kev). As the major element present, the K_α peak of iron accounted for approximately 50% of the total x-ray counts collected in scans of *Gallionella* stalks (Table 42). None of the above elements were detected in significant quantities when an x-ray energy scan was performed on the naked membrane surface adjacent to a stalk.

Figure 30b shows a highly magnified view of a portion of the *Gallionella* stalk shown in Figure 30a. An elemental map for iron is shown for the same section recorded between energy windows 6.25 kev and 6.55 kev (K_α for iron = 6.40 kev). The topological coincidence of the dots indicates the association of this element with the encrustations which envelope the stalk. A similar but lower intensity map was obtained for silicon for the same section indicating a close association of these elements in the stalk.

X-ray energy scans were also performed at different locations along individual *Gallionella* filaments. The data for three such filaments are summarized in Table 42. There were no significant changes in the ratio of the elements aluminum, silicon, phosphorous, calcium, iron, and zinc between different locations along the stalk. Nor were any significant changes in the same elemental ratios noted between stalks. These data suggest that the metabolic capacity of different *Gallionella* filaments to precipitate oxidized iron compounds was relatively uniform, and that the stoichiometry of mineral precipitation along a given filament was also uniform. Slight changes in the above elemental ratios might reflect possible chemical differences in the microhabitats which different *Gallionella* cells occupy *in situ*. Each of these microniches might be characterized by slightly different ionic concentrations of iron, silicon, and aluminum species. Conceivably, small changes in the *in situ* concentrations of specific ions might influence the overall stoichiometry of mineral oxidation and deposition on the cell surface.

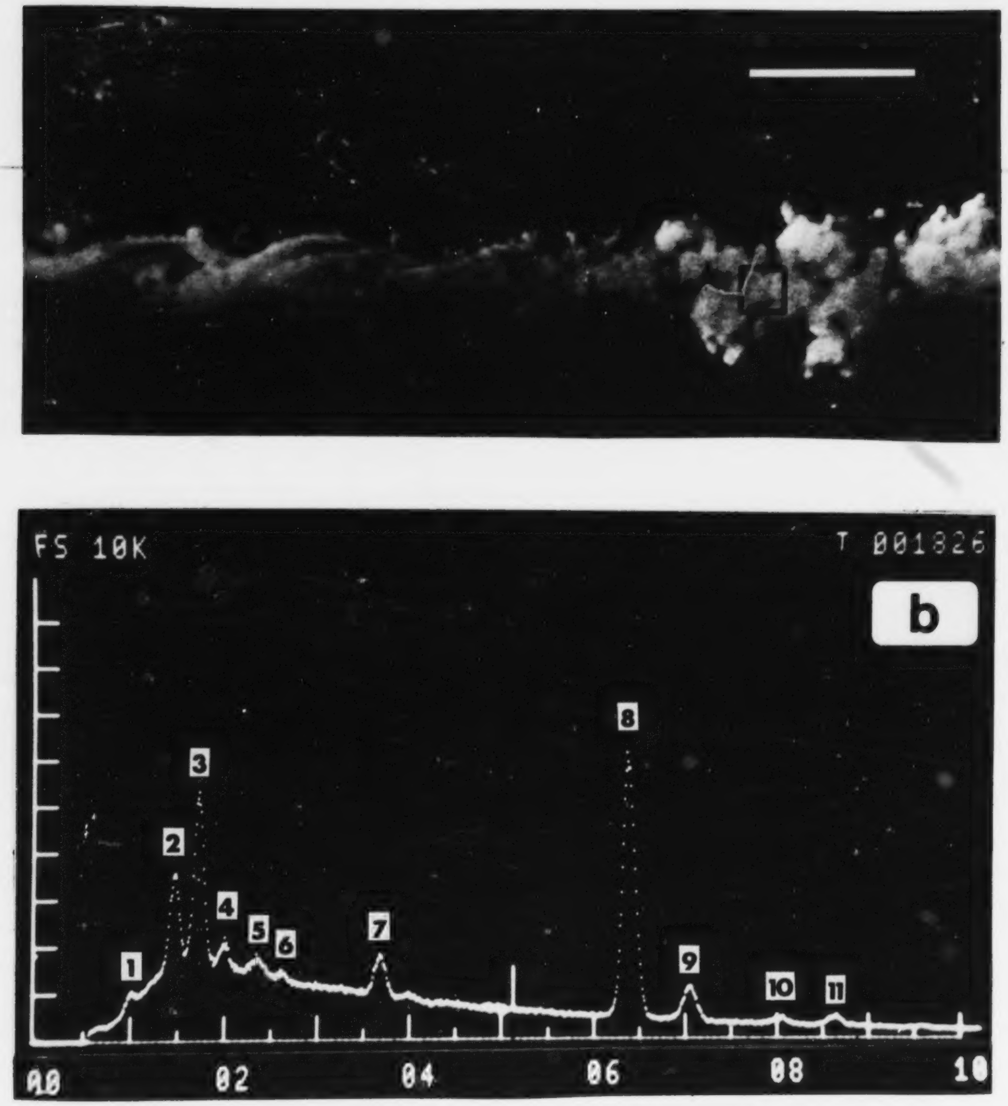

Figure 29. Scanning Electron Micrograph and Energy Dispersive X-Ray Analysis of Gallionella Stalks.

X-ray energy scan (b) of region of Gallionella stalk indicated in a. Peaks were identified as follows: peak 1, L_α line of zinc (1.001 kev); peak 2, K_α line of aluminum (1.49 kev); peak 3, K_α line of silicon (1.74 kev); peak 4, K_α line of phosphorous (2.01 kev); peak 5, K_α line of sulfur (2.31 kev); peak 6, K_α line of chlorine (2.62 kev); peak 7, K_α line of calcium (3.69 kev); peak 8, K_α line of iron (6.40 kev); peak 9, K_β line of iron (7.06 kev); peak 10, K_a line of copper (8.04 kev); peak 11, K_α line of zinc (8.64 kev). X-ray counts were integrated over a period of 1826 seconds. Bar = 3.0 μm.

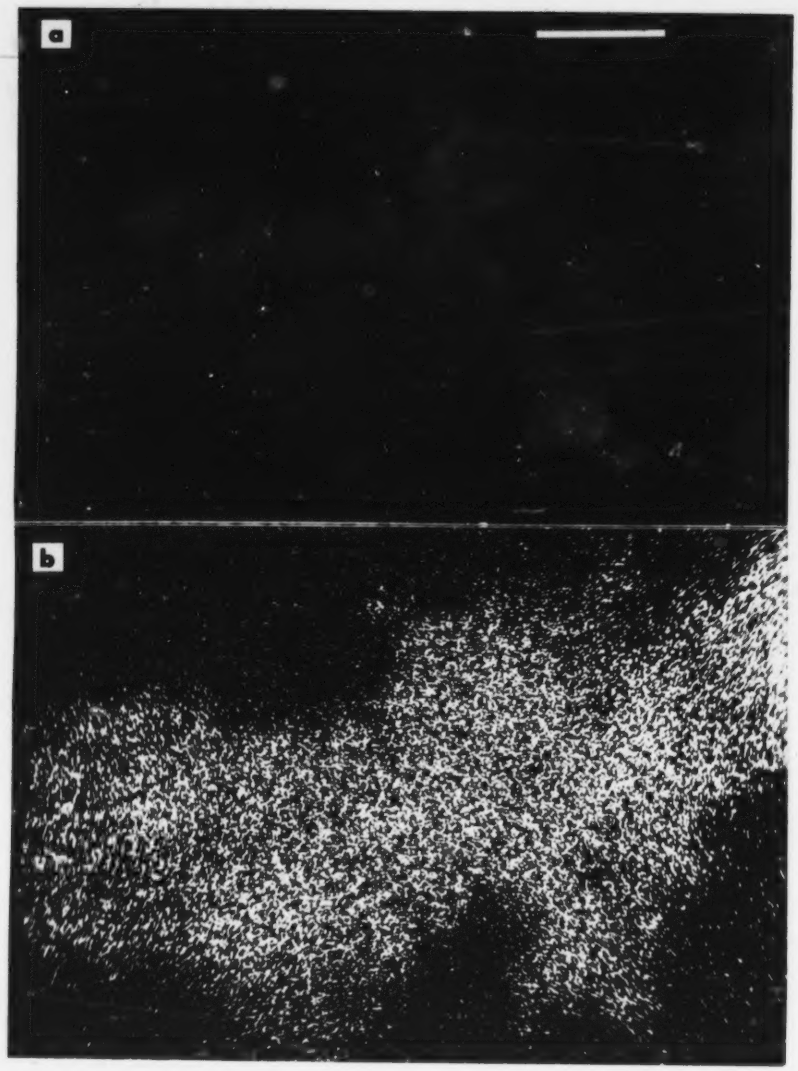

Figure 30. Elemental Map of Gallionella Stalk.

X-ray energy map for iron (b) for portion of Gallionella filament shown in a (box). This is the same Gallionella stalk as shown in Figure 3a. X-ray counts were collected for 420 seconds between energy windows 6.25 kev and 6.55 kev (K_α energy line for iron = 6.40 kev). Bar = 1.0 μm.

TABLE 42. RATIOS[1] AND PERCENT X-RAY INTENSITIES (IN PARENTHESES) OF SELECTED ELEMENTS IN GALLIONELLA STALKS[2]

Location of X-ray Scan Along Stalk:

Element	Left Terminus[3]	Middle	Right Terminus
	$\overline{x}$ ± S.D.	$\overline{x}$ ± S.D.	$\overline{x}$ ± S.D.
Al	.177 ± .039	.177 ± .001	.210 ± .094
	(8.92 ± 1.97)	(9.15 ± .825)	(9.71 ± 3.95)
Si	.474 ± .119	.440 ± .064	.449 ± .088
	(23.6 ± 3.87)	(22.6 ± 1.78)	(22.0 ± 2.19)
P	.099 ± .052	.103 ± .064	.101 ± .060
	(4.86 ± 2.43)	(5.15 ± 2.96)	(4.91 ± 2.58)
Ca	.112 ± .032	.097 ± .032	.125 ± .042
	(5.58 ± 1.43)	(4.92 ± 1.45)	(6.13 ± 1.70)
Fe (K_α)	1.000 ± 0.0	1.000 ± 0.0	1.000 ± 0.0
	(50.5 ± 4.77	(51.8 ± 4.12)	(49.8 ± 5.73)
Fe (K_β)	.1254 ± .0512	.121 ± .009	.129 ± .012
	(6.302 ± .612)	(6.24 ± .044)	(6.48 ± 1.32)
Zn	.013 ± .003	.012 ± .005	.020 ± .014
	(.680 ± .210)	(.599 ± .299)	(.902 ± .683)

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[1] All elemental ratios were obtained by normalizing the intensity of the element to the intensity of the K energy line iron.

[2] Arithmetic means and standard deviations are displayed for three different stalks.

[3] Left and right ends of stalks were arbitrarily selected.

This report is the first to describe the total elemental constitution of Gallionella stalks recovered directly from the environment. Extracellular ferro-alumino-silicate deposits coating the surfaces of Gallionella stalks have not been previously reported. Such complex mineral precipitates may not be formed on cells grown in relatively simple, chemically defined laboratory media. Only a few analytical studies have been done on the superficial encrustations of Gallionella cells grown in such media. Mardanyan and Balshova (79) investigated the state of iron on G. ferruginea stalks using microdiffraction, x-ray diffraction, and nuclear gamma-resonance spectroscopy. They found that the oxidized iron (Fe^{3+}) was apparently actively and stably combined with the surfaces of the proteinaceous fibrils which comprise the stalk. Since no diffraction rings were ever observed, the Fe^{3+} detected was probably not organized into any kind of crystalline matrix, which is known to occur in simple $Fe(OH)_3$ deposits. They concluded, therefore, that the oxidized metal may be intimately associated with the protein fibrils in "a structure quite different from that of known compounds of oxidized iron." The precise chemical structure of the ferro-alumino-silicate deposits described here is unclear, and it is not known how closely the other elements, such as Si, Ca, and Zn are associated with the protein components of the stalks. Further analytical studies may ultimately help to elucidate the precise structure of these biologically-mediated mineral complexes. Such information will be of value in understanding how these microorganisms chemically modify the pipe surface, making it more susceptible to corrosion processes and fouling by other microorganisms.

Scanning electron microscope evidence for bacterially-colonized particles in potable groundwater: occurrence and chemistry.

In addition to bacteria attached to pipe surfaces, large numbers of rod-shaped bacteria were also found adhering to the surfaces of suspended particulate matter recovered from water samples by filtration (Figures 31-34). Particles exhibiting an attached microflora were often cavitated and generally had a particle size diameter ranging from about 10 to 50 μm. Such particles were, for the most part, irregularly shaped and highly variable in surface texture. In the majority of instances, the bacteria were clearly attached in a superficial fashion to the outside of the particle but often appeared to be covered by an extracellular slime layer (Figures 31-34). Biosynthesis of similar extracellular mucopolysaccharide substances by Pseudomonas aeruginosa has been shown to be an important factor in decreasing the susceptibility of this microorganism to chlorine disinfection (111). In other instances, the cells were entrained within a mass of extracellular fibrillar or mucoid appearing material (Figures 33, 34).

The total number of particles in the 10 to 50 μm size range fluctuated considerably between sampling times and locations. Quantitative SEM observations usually indicated between 100 to 1000 particles of this size range (i.e., 10 to 50 μm) per ml of water sample. Typically, the majority of these particles were not colonized by bacteria. For example, at one sampling location and time (well site #6064, 15 May, 1979) the number of particles in the 10 to 50 μm size category recovered on membrane filters corresponded to an in situ concentration of approximately 900 particles per ml. Approximately 17% of these, or about 150 particles per ml, were observed to bear attached bac-

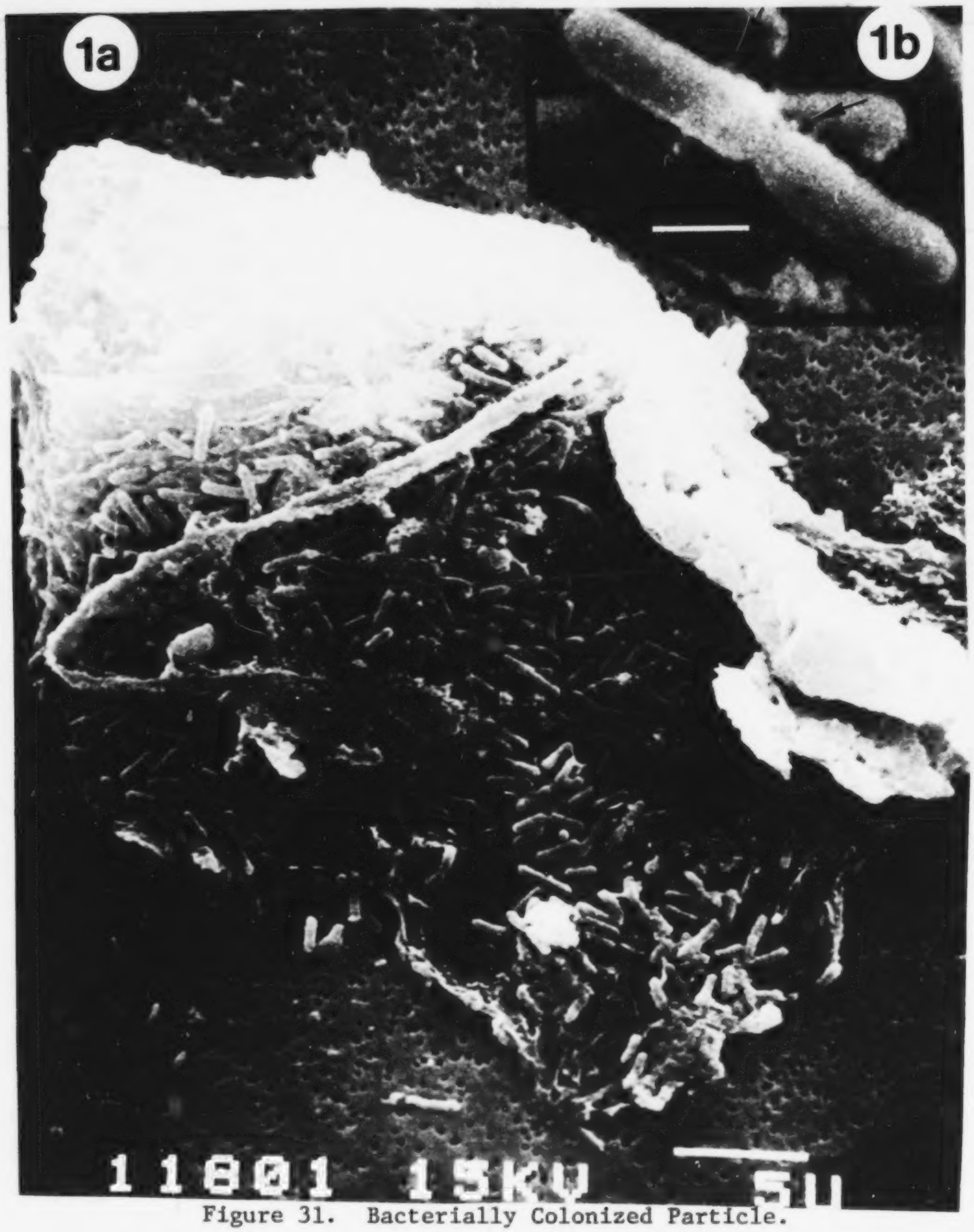

Figure 31. Bacterially Colonized Particle.

(a) Scanning electron micrograph showing a bacterially-colonized particle recovered from well site #6321. Approximately 320 rod-shaped bacterial cells are visible. Note that the majority of the cells appear to be superficially attached without extensive glycocalyx formation. Bar = 5.0 μm.
(b) High magnification view of area delineated in (a). Arrows indicating rudimentary glycocalyx formation. Bar = 5.0 μm.

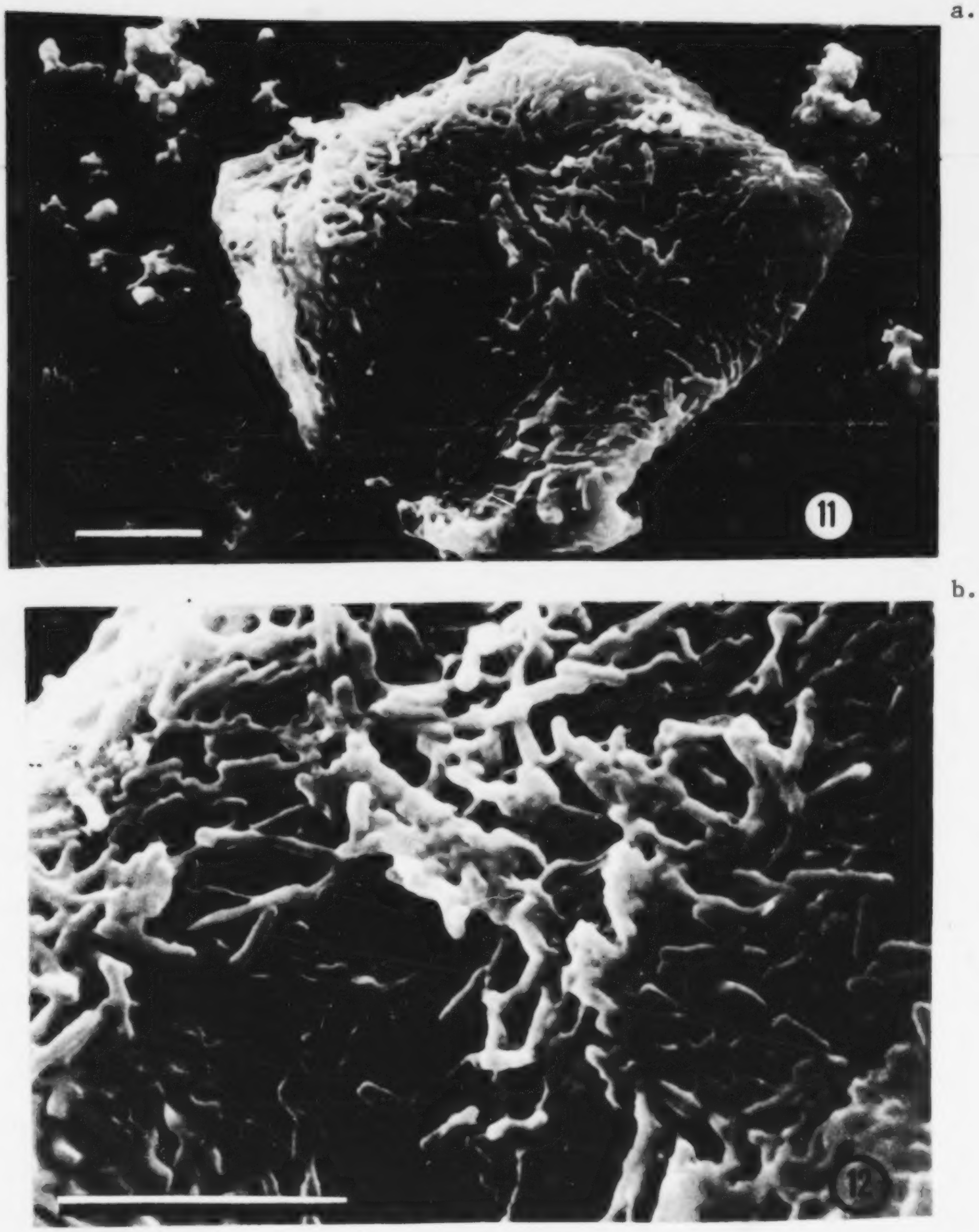

Figure 32. Bacterially Colonized Particle.

(a) Particle recovered from well site #6064 bearing numerous attached bacilli. Note that many cells appear to be enveloped by a layer of extracellular slime or capsular material (arrows). Bar = 5.0 μm.

(b) Close-up view of attached cells shown in Figure 32a. Bar = 5.0 μm.

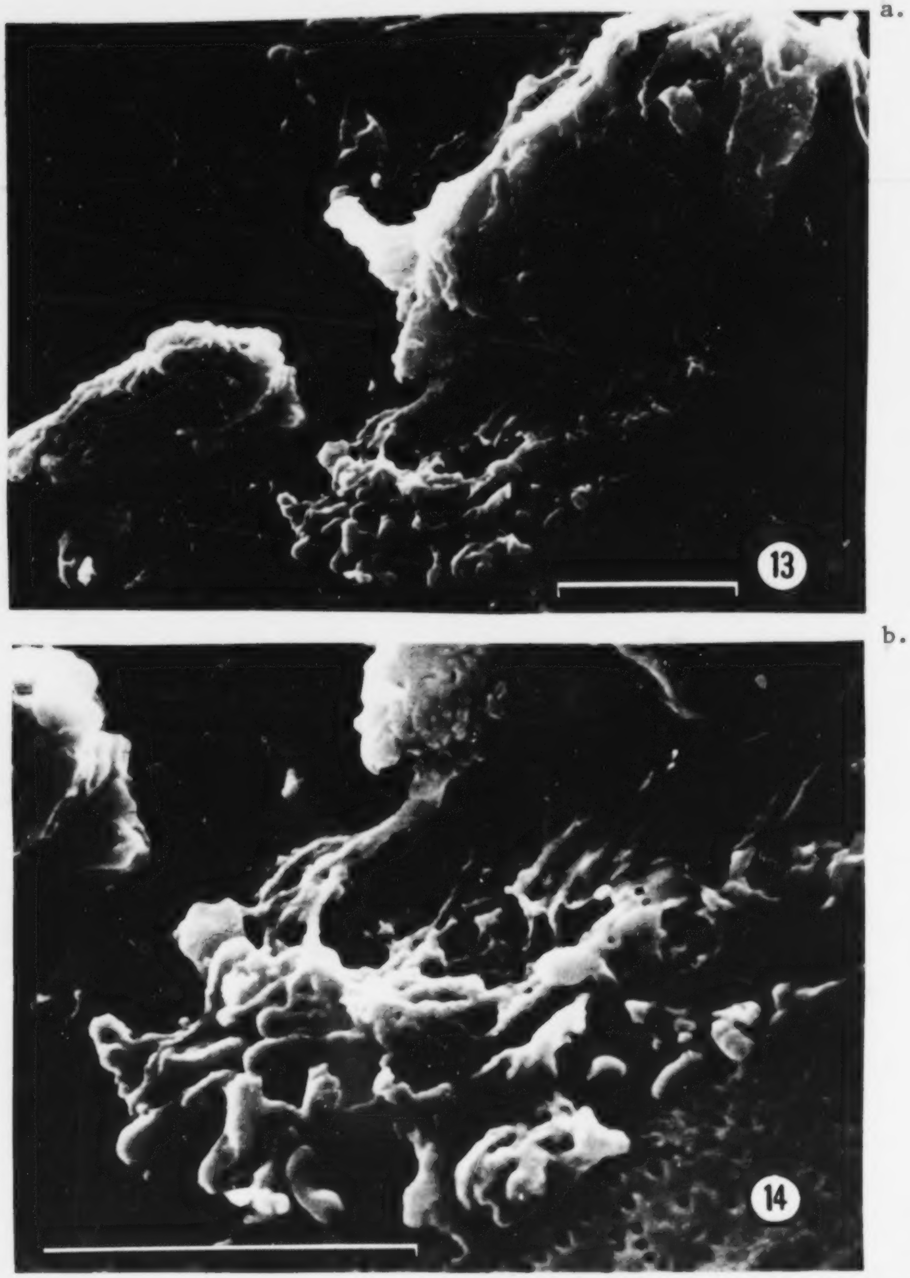

Figure 33. Bacteria Encased in Mucilageous Substance on a Particle.

(a) Particle recovered from well site #6064 showing attached bacteria (arrows) entrained in mucilaginous substance. Bar = 5.0 µm.

(b) Close-up view of attached cells shown in Figure 33a. Bar = 5.0 µm.

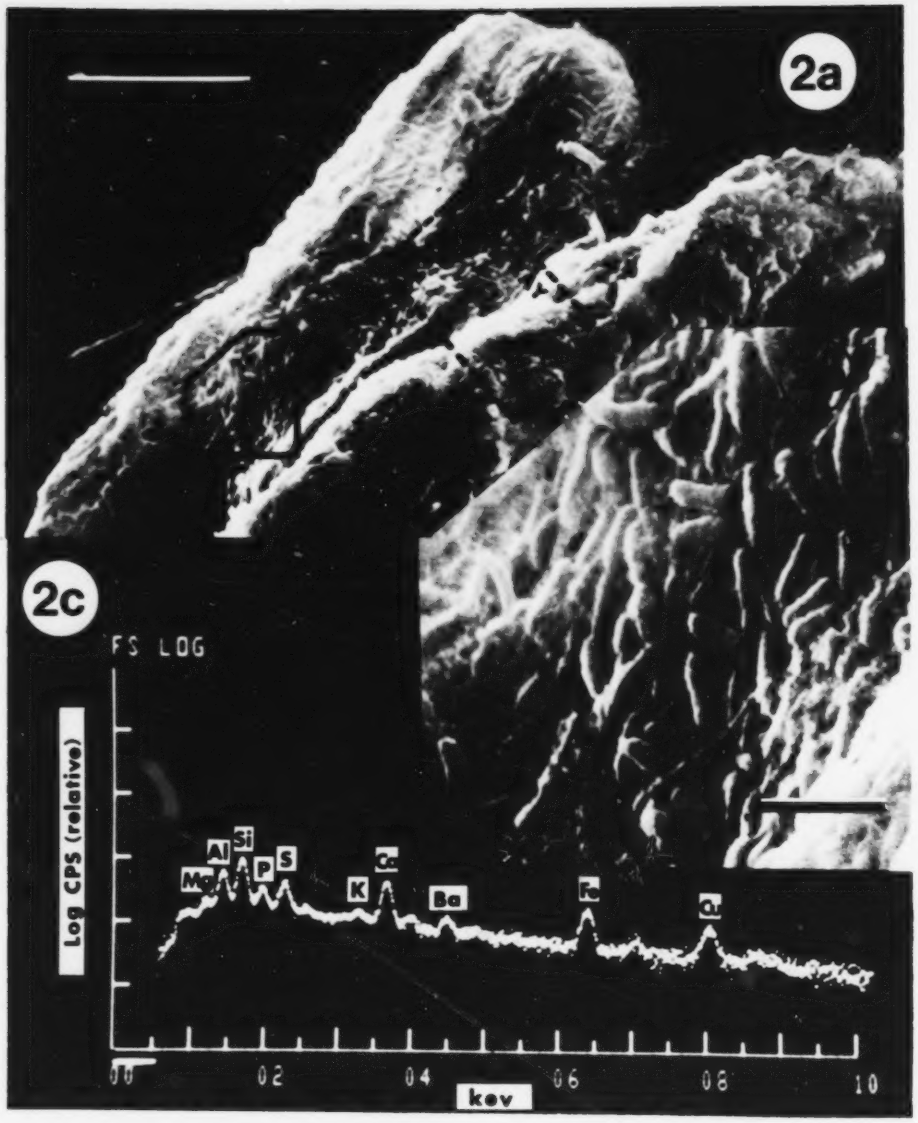

Figure 34. Scanning Electron Micrograph and Energy Dispersive X-Ray Analysis

(a) Scanning electron micrograph of a relatively large bacteria-laden particle recovered by filtration from well site #6321. Carbon-coated specimen. Bar = 30 m.
(b) High magnification view of area enclosed by solid lines in a. Many of the rod-shaped cells appear to be partially occluded from view, presumably by bacterially-secreted extracellular mucopolysaccharide material. Bar = 5.0 m.
(c) X-ray energy dispersive microanalysis spectrum from 0 to 10 kev of area of particle delineated by broken lines in a. A total of ten distinct x-ray energy manimum (maxima) are visible in the spectrum indicating the presence of 8 different elements. X-ray counts were integrated over a period of 200 seconds.

teria, usually all of one morphological type. Since 10 to 100 bacteria were counted on each particle, a milliliter of water could contain from 1,500 to 15,000 particle-bound bacterial cells.

Conceivably, the chemical nature of the suspended particulate matter could strongly influence the ability of certain bacteria to attach to and multiply on particles. Therefore, the complete elemental composition of both colonized and uncolonized particles was examined using EDX (Figure 34). Elemental analyses of a total of 693 suspended particles ranging in size from 2.0 μm to 50.0 μm in diameter were performed using the coupled x-ray scan image analysis system described above. (No particles in the > 30 μm size range were detected). Many of the elements were chemically and morphologically similar (Table 43). The major elements were Al, Si, S, Ca, Ba, and Fe. Smaller quantities of Ni, Cu, Co, Ti, P, K, and Zn were also frequently detected. Elements representing less than 10% of the x-ray composition of the total particle population (i.e., the 693 particles) included: Mg, Va, Cr, Mn, Hg, Pb, Se, and Br. Approximately 20% or greater of the particles encountered in this study may possess a largely organic composition. The remainder of the particles are primarily inorganic and probably correspond to specific mineral substances. It is possible that some of the inorganic type particles could enter the water column during bacteriological sampling by breaking off from the mineral concentrations which coat the sides of the pipe or reservoir walls. Particles which appeared on visual inspection to be primarily organic in nature often were found to contain traces of the elements S, Cl and K. Particles of a totally organic nature such as detritus were readily detected since they failed to emit detectable x-rays when bombarded by the electron beam and represented approximately 3.9% of the total particles examined (Table 43). Bacteria were observed attached to both inorganic and organic type particles.

X-ray analyses of unattached bacterial cells lying free on the Nuclepore membrane surface frequently produced spectra indicating the presence of the elements P, Cl and S. Infrequently, one or two of these elements were not readily detected in the bacterial cells, presumably due to limitation in instrument sensitivity. Bacterially-colonized particles nearly always exhibited x-ray energy spectra characteristic of the elements Al, Si, P, Cl, Ca, S, and Fe suggesting that bacteria were preferentially associated with aluminosilicates of calcium and iron enriched particles. Utilizing this information three distinct hypothetical classes of bacterially-colonized particles were provisionally adopted, based on the presence of these seven elements. In order to be categorized as a bacterially-colonized particle, the elements P, Cl and S, which are known to be present in unattached bacterial cells, must be detected, as well as those additional elements in colonized particles (i.e., Al, Si, Ca and Fe). Approximately one percent of the 693 particles analyzed contained all seven of these elements and were collectively designated as theoretically colonizable Class #1. Another 7.5% of the particles were composed of Al, Si, Ca and Fe and, in addition, contained two (Class 2) or one (Class 3) of the elemental constituents found in the unattached bacterial cells (i.e., P, Cl or S). The relative probability of a particular class particle being colonized by bacteria would then decrease in the order Class #1 > Class #2 > Class #3. Suspended particulate matter containing no P, Cl or S were assumed to be completely devoid of microorganisms and, as such, could

TABLE 43. DESCRIPTION OF 693 PARTICLES (2.0μm to 50.0μm) FROM WELL 6321 ANALYZED BY COMPUTER ASSISTED SCANNING ELECTRON MICROSCOPE ENERGY DISPERSIVE X-RAY ANALYSIS

Class	Population %	Relative Elemental (%) Contribution to x-ray count*
No/Low x-rays	3.9	≥ 96% organic
Theoretically Colonizable**	8.4	Si=34, Fe=17, Ca=13, Al=11, S=6, Cl=6, P=3
Barite	17.5	Ba=66, S=33
Apatite	4.6	Ca=59, P=26, Al=9
Calcium Carbonate	7.8	Ca=96
Silica-Calcium	2.4	Ca=47, Si=33, Al=6, S=4, Fe=3
Heavy Metal Rich	7.5	Fe=25, Si=22, Cu=18, Al=10, Ca=10
"ILLITE"	1.6	Si=57, Al=23, K=6, Fe=7, Ca=3
"Kaolinite"	4.0	Si=49, Al=48, Na=1
Other Alumino-silicates	12.5	Si=60, Al=16, Ca=8, K=5, Fe=4
Iron Rich	2.0	Fe=55, Si=30, Al=11, Ca=2, Mg=2
Chlorine Rich	8.8	Cl=64, Na=20, K=8, Ca=5
Sodium Rich	1.4	S=29, Na=27, Cl=13, Si=10, Al=7, Ca=6
Silicon Rich	3.6	Si=83, Al=4, Ca=4, Na=3, K=2, Fe=1
Silicon dioxide	4.9	Si=97, Na=2
Miscellaneous	9.1	Fe=27, Si=26, Al=12, K=8, Ca=7, Ti=5

*Only x-rays from elements of atomic number 11 (Na) and above are added into the total x-ray count. Data should not be interpreted as elemental composition.

**A combination (weighted average) of Class 1, 2, and 3. See text.

cal agent. Though hypothetical in nature, evidence has been presented which suggests the possibility of transmission of disease through the ingestion of waterborne bacterially colonized particles. Therefore, understanding the occurrence of bacteria-laden suspended particulate matter and the factors that influence bacterial attachment to particles, both in untreated groundwater supplies and water distribution systems, would be of primary public health significance.

Chlorine Resistance

Physico-chemical and bacteriological properties of distribution systems. The major physico-chemical characteristics of the influent (source) water and distribution system water (from selected hydrant locations) for the cities of Irvine and Garden Grove are shown in Table 44. Both waters were of high quality according to current standards, similar in mineral composition, and of relatively low turbidity. The most significant difference between the two systems for the purposes of the present investigation was the maintenance of a free available chlorine residual (i.e., $HOCl = OCl^-$) of about 0.6 to 0.7 mg l^{-1} throughout the Irvine system. Virtually no free chlorine was present in the Garden Grove system. At the pH values of the Irvine system water, approximately 40% or about 0.24 to 0.29 mg l^{-1}, of the measurable free available chlorine was present as un-ionized hypochlorous acid (HOCl), which is strongly bacteriocidal (95). Lower concentrations of chloramines and chlorinated derivatives of a variety of organic compounds may also occur in the Irvine system, since significant quantities of ammonia nitrogen (0.02 to 0.03 mg l^{-1}), Kjeldahl nitrogen (0.17 to 0.40 mg l^{-1}), and total organic carbon were routinely detected. However, because of their low concentrations and reduced toxicities compared to uncombined hypochlorous acid (95), the chloramines and chlorinated organic substances formed should not contribute appreciably to the total bacteriocidal activity of the water. Inorganic ions which can potentially interfere with *in situ* chlorine measurements, such as the oxide forms of manganese and iron, were not present in sufficiently high concentrations to cause measurement interferences (Table 44).

Despite consistently high levels of free available chlorine (above 0.5 mg l^{-1}). throughout the Irvine distribution system, large numbers of bacteria were regularly isolated from water samples (Figure 35). Whereas the *in situ* concentration of free chlorine correlated negatively with the total number of viable bacteria (as determined by standard plate count procedures), the Pearson r correlation coefficient relating these parameters was not statistically significant ($r = -0.089$). Numerous instances occurred in the Irvine system where greater than 500 bacteria per ml were recovered from water containing more than 0.6 mg l^{-1} free available chlorine. These data strongly suggest that certain bacteria may possess mechanisms which enable them to survive in highly chlorinated waters.

Standardization of the disc assay. Since a number of physico-chemical parameters influenced the size of the inhibition zone produced in the disc assay, rigorously standardized assay conditions were necessary. The most significant factors affecting zone size were: (i) the chlorine demand of the growth medium, (ii) the concentration of sodium hypochlorite applied to the represent a fourth and separate class. From this preliminary interpretation of the data, bacterially-colonized particles theoretically could comprise approximately 8.5% of the total particle population. Scanning electron microscope studies of groundwater samples from the same distribution system showed that 1 to 17% of all suspended particulate matter was colonized by bacterial cells. Many particles were observed which, although not colonized, apparently possessed an elemental spectrum theoretically suitable for bacterial attachment. This overlap in the chemical similarities of the colonized and uncolonized particles suggests that bacterial adhesion to suspended particulate matter is a highly variable phenomenon in the environment not limited solely by the chemical composition of the particles themselves, but also by other as yet unknown physico-chemical and biological factors.

Bacteria adhering to non-living mineral or detritus particles have been detected in a wide variety of natural aquatic environments, including mountain streams (42), river water, lake water (13) and sea water (80, 94, 114). Bacterial cells attached to free floating (planktonic) detritus particles were found at all sampling depths in Lake Tahoe (U.S.A.) and are believed to be primarily responsible for detrital aggregation and decomposition (94). These sessile populations of microorganisms have been shown to be metabolically active and contribute significantly to the total microheterotrophic activity of certain aquatic ecosystems (13, 80). Studies of the kinetics of uptake of specific radiolabeled organic compounds by bacteria attached to particles and pipe surfaces may ultimately help to elucidate how metabolically active these cells are compared to unassociated cells that remain freely suspended in the water column.

This report is the first to describe the chemical composition and occurrence of bacterially-colonized particles in untreated potable groundwater. In 1970, groundwater supplies provided approximately 19% of the water used for human consumption in the United States and 47% of that used in the Western United States (41). Groundwater has repeatedly been a source of waterborne disease outbreaks, many with no apparent etiological agent. During the period of 1971 to 1977, a total of 192 waterborne disease outbreaks occurred in the United States. No etiological agent was detected in the water supply in 57% of these outbreaks. Approximately 35 such outbreaks occur in the United States per year compared to 23 per year reported between 1920 and 1935. Approximately 49% of outbreaks and 42% of illnesses were due to the use of untreated, improperly treated or contaminated groundwater. Untreated groundwater accounted for approximately 10% of the reported cases of illness and untreated surface water for 14% of the cases. The majority of the remaining outbreaks were explained by treatment failure or deficiencies. In semi-public water systems, untreated groundwater accounted for 43% of the outbreaks (28). Diseases caused by these waterborne outbreaks include chemical poisoning, giardiasis, shigellosis, hepatitis, salmonellosis, typhoid fever and enterotoxigenic diarrhea. The remianing disease outbreaks were catagorized as acute gastrointestinal illness (28).

The data presented in this paper clearly indicate that large numbers of bacteria could be ingested on particles in a small volume of water. The sporadic occurrence of such colonized particles would make it difficult to trace the origin of a disease in drinking water or to delineate the etiologi-

TABLE 44. MAJOR PHYSICO-CHEMICAL PROPERTIES OF IRVINE AND GARDEN GROVE WATER[1]

		Irvine System				Garden Grove System			
Parameter	Unit	Source[2]		Site[3]		Source[4]		Site[5]	
Free Chlorine	mg/1	0.73	0.14	0.61	0.15	0.005	0.003	0.03	0.06
pH		7.77	0.44	7.7	0.58	7.22	0.53	7.39	0.43
Temperature	°C	17.5	4.96+1	19.8	4.72	18.9	1.62	19.9	2.4
Kjeldahl-N	mg/1	0.4	0.09	0.17	0.12	0.005	0.001	0.07	0.09
Ammonia-N	mg/1	0.03	0.03	0.02	0.02	0.02	0.04	0.04	0.01
Turbidity	NU	0.42	0.19	2.3	7.44	0.26	0.11	6.61	26.2
Electroconductivity		836.0	246.0	955.0	294.0	629.0	198.0	993.0	335.0
Redox	mV	288.0	142.0	222.0	151.0	137.0	43.3	97.9	87.3
Dissolved oxygen	mg/1	9.69	1.39	9.23	1.49	7.62	0.9	7.17	0.99
Sodium	mg/1	86.6	3.84	85.7	3.31	40.1	6.77	54.8	8.08
Potassium	mg/1	4.93	0.28	4.88	0.35	3.86	0.45	4.39	0.35
Calcium	mg/1	57.5	5.43	60.0	9.48	81.3	9.03	89.2	16.8
Magnesium	mg/1	25.0	2.36	23.3	7.12	15.9	3.23	21.4	8.55
Manganese	mg/1	3.5	3.82	5.75	4.81	3.65	4.14	9.3	8.56
Iron	mg/1	26.5	19.2	12.2	12.3	20.0	30.4	239.0	326.0
Silicon	mg/1	7.53	6.27	17.7	21.0	8.72	10.9	11.3	12.2
Cobalt	mg/1	1.14	0.86	1.19	0.98	1.44	1.94	1.50	1.43
Copper	mg/1	17.0	45.2	6.81	4.02	56.0	173.0	10.6	8.47
Zinc	mg/1	4.57	2.9	4.88	3.76	46.1	97.2	7.68	7.72
Bicarbonate	mg/1	124.0	7.18	125.0	7.06	215.0	9.63	215.0	23.7

(continued)

TABLE 44 (continued)

		Irvine System				Garden Grove System			
Parameter	Unit	Source[2]		Site[3]		Source[4]		Site[5]	
Carbonate	mg/l	0.66	0.27	0.72	0.29	0.7	0.43	0.65	0.26
Carbon dioxide	mg/l	2.49	1.05	2.29	1.3	8.98	7.58	8.25	7.99
Chloride	mg/l	84.7	8.08	83.2	7.6	58.2	65.8	66.9	10.4
Sulfate	mg/l	206.0	21.1	203.0	24.6	94.2	19.5	139.0	33.3
Nitrate	mg/l	0.78	0.65	0.93	0.68	17.6	11.7	18.7	8.9
Orthophosphate	mg/l	38.0	53.5	42.8	58.5	40.8	51.9	43.3	59.6

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[1] Arithmetic means and standard deviations of bimonthly data collected from 6/78 to 4/80.

[2] Irvine source (hydrant) data.

[3] Paseo Picasso St. (hydrant) data.

[4] Data from well/reservoir sites 6064, 23/9, 6321.

[5] Rockinghorse and Harris St. (hydrant) data.

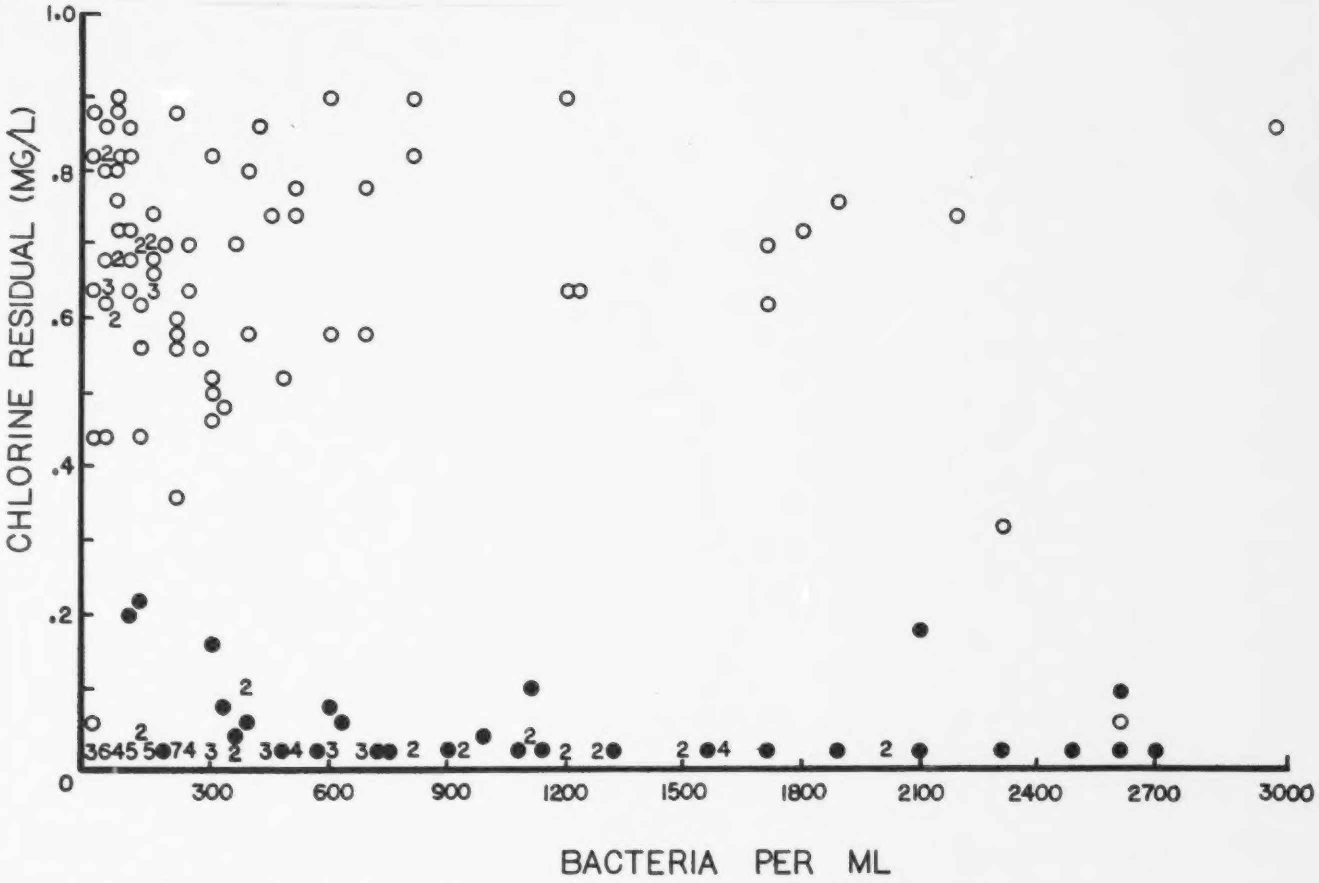

Figure 35. Relationship Between Bacterial Colony Recovery on M-SPC Medium and Redicual Chlorine

Number of bacterial colonies recovered on M-SPC medium as a function of the in situ concentration of free available chlorine. Bimonthly data collected from 6/78 through 4/80. Symbols: 0 = Irvine data; ● = Garden Grove data.

disc, and (iii) the number of bacterial cells applied to the surface of the medium.

The organic-nitrogen concentration of the disc assay broth was approximately 213 mg N 1^{-1}, while the ammonia nitrogen concentration was about 6.1 mg N 1^{-1}. This high organic-nitrogen concentration was reflected in the shape of the dose-residual ("breakpoint") curve for the medium (Figure 36). Since the reactions of chlorine with ammonia are usually completed rapidly at pH 7.0, the persistence of a combined residual indicated the presence of relatively stable chlorinated organic-nitrogen compounds (95). Although the presence of such chlorinated organic compounds precluded an accurate breakpoint determination, a marked inflection occurred at about 1900 mg 1^{-1} applied chlorine. Since the ratio of organic to ammonia nitrogen in the medium was about 35:1, comparatively small quantities of free monochloramine were produced. Because the medium exhibited a large molar excess of chlorine demand compared to the amount of chlorine applied to the discs, it was concluded that the inhibition zone resulted from the diffusion outward from the discs of a complex mixture of chloramines and stable chlorinated organic-nitrogen compounds which exhibit bacteriocidal activity. The specific chemical identities of the chloroderivatives were not investigated.

An increase in the chlorine demand of the medium led to a marked decrease in the sizes of the inhibition zones (Figure 37), presumably because of a dilution effect of the added chlorine at higher demands. Nearly all of the bacterial isolates tested grew well on a medium containing 0.5 g 1^{-1} each of tryptone, yeast extract, and glucose. Since this concentration of organics was also sufficiently low to allow the formation of relatively large inhibition zones, it was adopted for use in the standardized disc assay (see Materials & Methods).

There was an approximately linear relationship between the concentration of sodium hypochlorite applied to the discs and the inhibition zone diameters (Figure 38). While each isolate was routinely tested using four different applied sodium hypochlorite concentrations (0, 10, 20, and 40 μm per disc), generally data obtained only from the disc containing the highest concentration of halogen was used to compare zone sites.

Bacteria themselves exert a significant chlorine demand; thus, it was of interest to examine the effect of cell number as a function of zone size. These data, shown in Figure 39, indicate that a small (4.3 mm) though noticeable increase in the mean zone diameter resulted when the number of bacteria in the inoculum was decreased from 1.8 x 10^7 per ml to 3.7 x 10^4 per ml (500-fold dilution). Therefore, prior to plating the bacteria, each experimental culture was routinely adjusted to a barely visible optical turbidity ($OD_{580\ nm}$ = 0.01 to 0.02). This corresponded to a cell number of approximately 7.3 x 10^6 to 1.8 x 10^7 per ml. This source of variability in inoculum size had a negligible effect on the size of the inhibition zone produced.

Each bacterial isolate tested in the disc assay exhibited a characteristic and reproducible zone size. In Figure 40 are shown examples of disc tests for three different bacterial isolates. The order of sensitivity to organically-combined chlorine for the three organisms shown was Pseudomonas

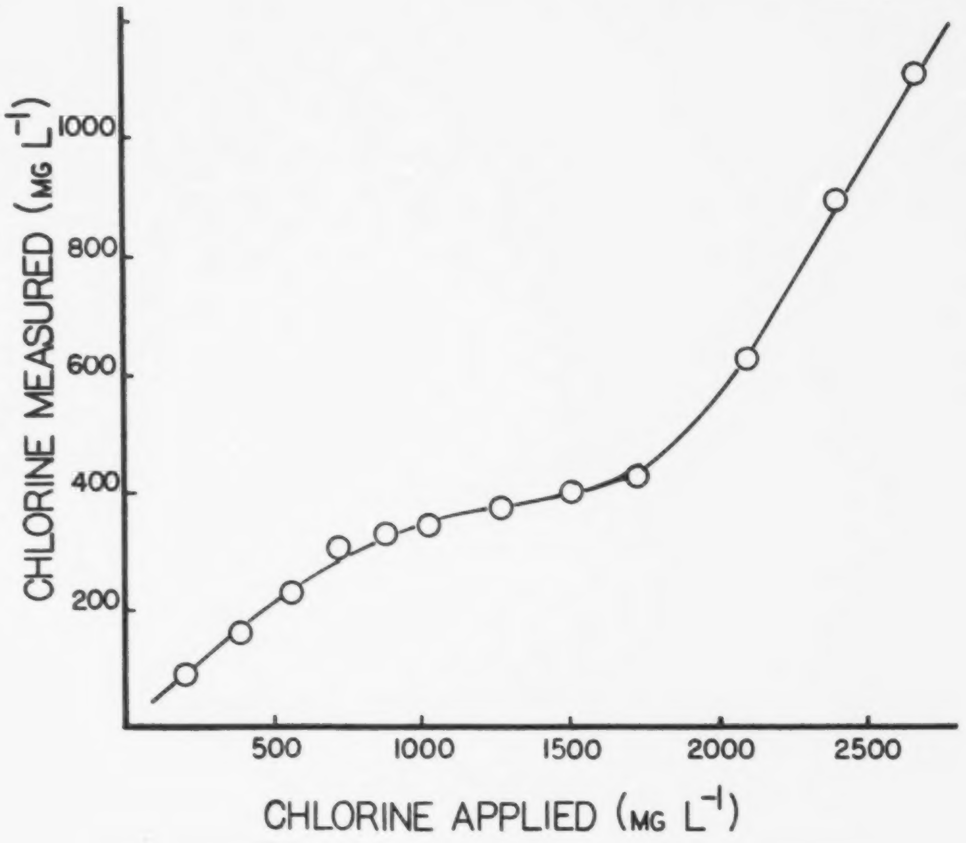

Figure 36. Concentration of Free Available Chlorine as a Function of Applied Chlorine in Disc Assay Broth.

Concentration of free available chlorine (determined amperometrically) as a function of the applied chlorine concentration for disc assay broth. Free chlorine appears at a concentration of approximately 1900 mg l^{-1} applied chlorine.

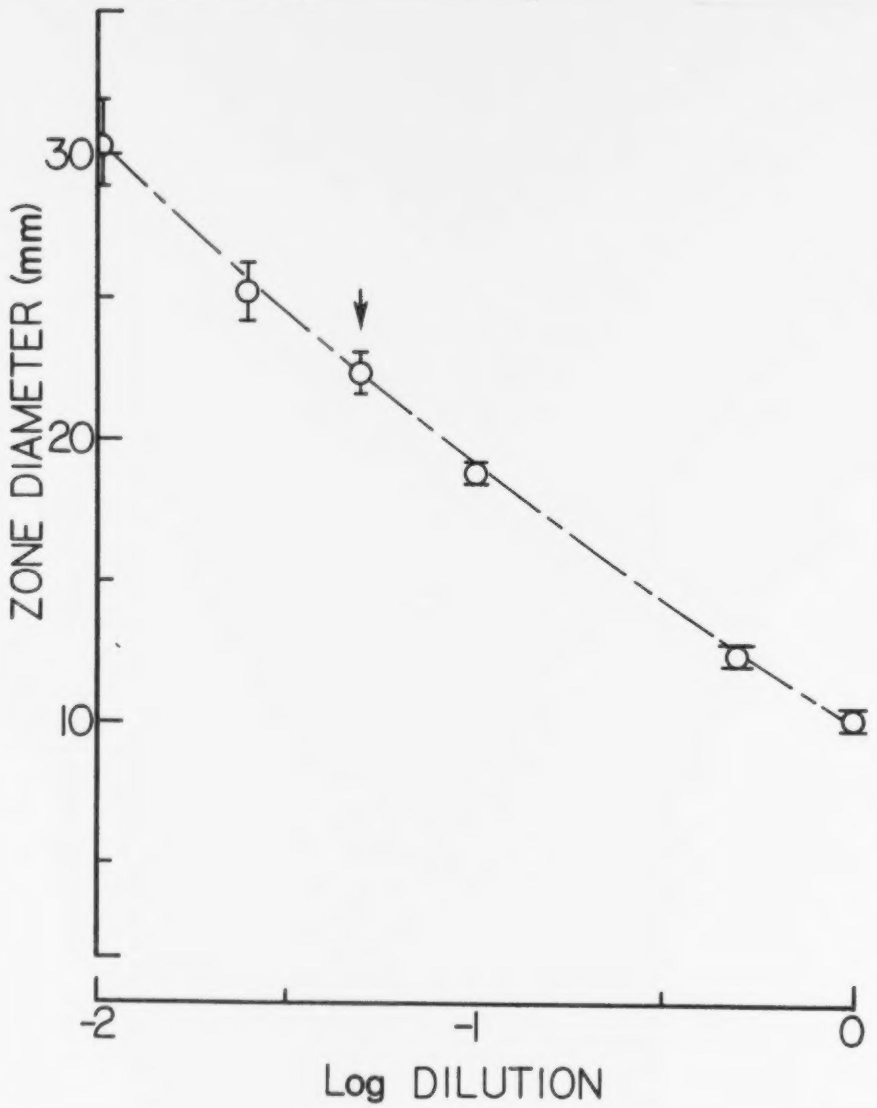

Figure 37. Inhibition Zone Diameter for *E. coli* as a Log Function of Assay Agar Dilution

Inhibition zone diameter as a function of the log of the dilution of a medium containing (per liter of distilled water): glucose, 10 g; yeast extract, 10g; tryptone, 10 g. Arrow indicates the strength of the medium used in the standard disc assay, 0.5 g each of the above components. All media contained 15.0 g of Difco agar. Each data point represents N = 5 replicates. Forty micrograms of sodium hypochlorite were applied to each disc. Cells: *E. coli* strain #40

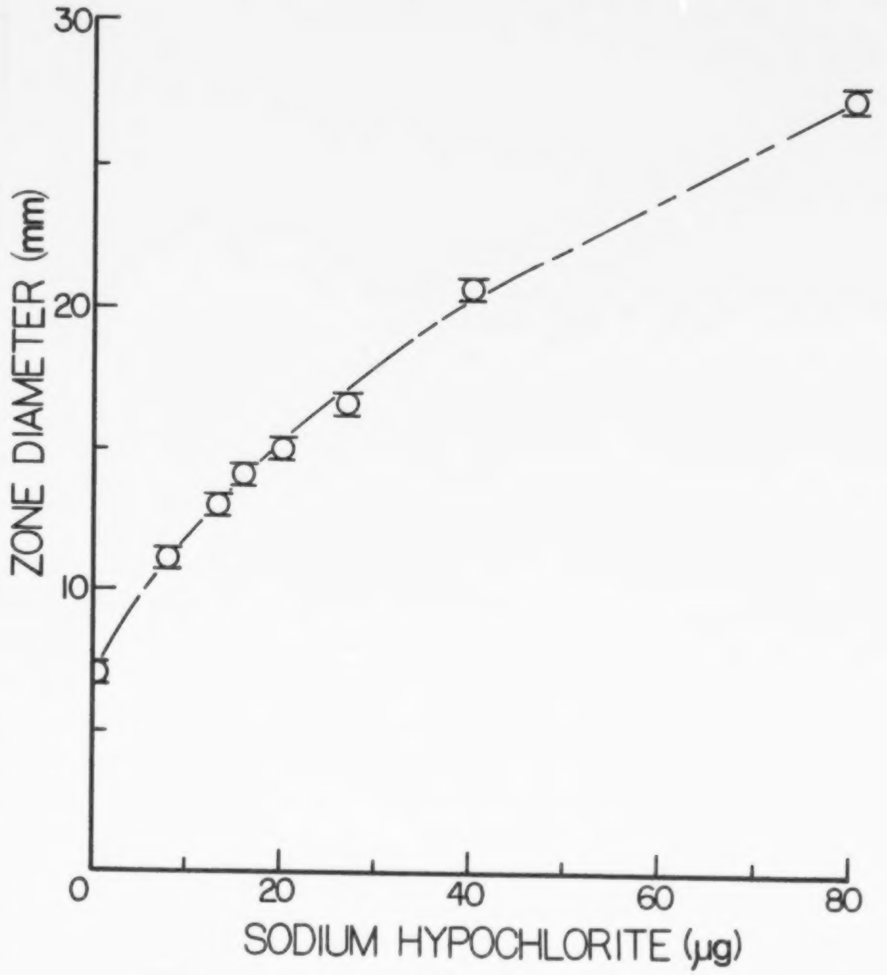

Figure 38. Inhibition Zone Diameter as a Function of Applied Sodium Hypochlorite

Inhibition zone diameter as a function of the amount of sodium hypochlorite applied to the disc. Each data point represents N = 4 to N = 9 replicates. Cells: *E. coli* strain #40.

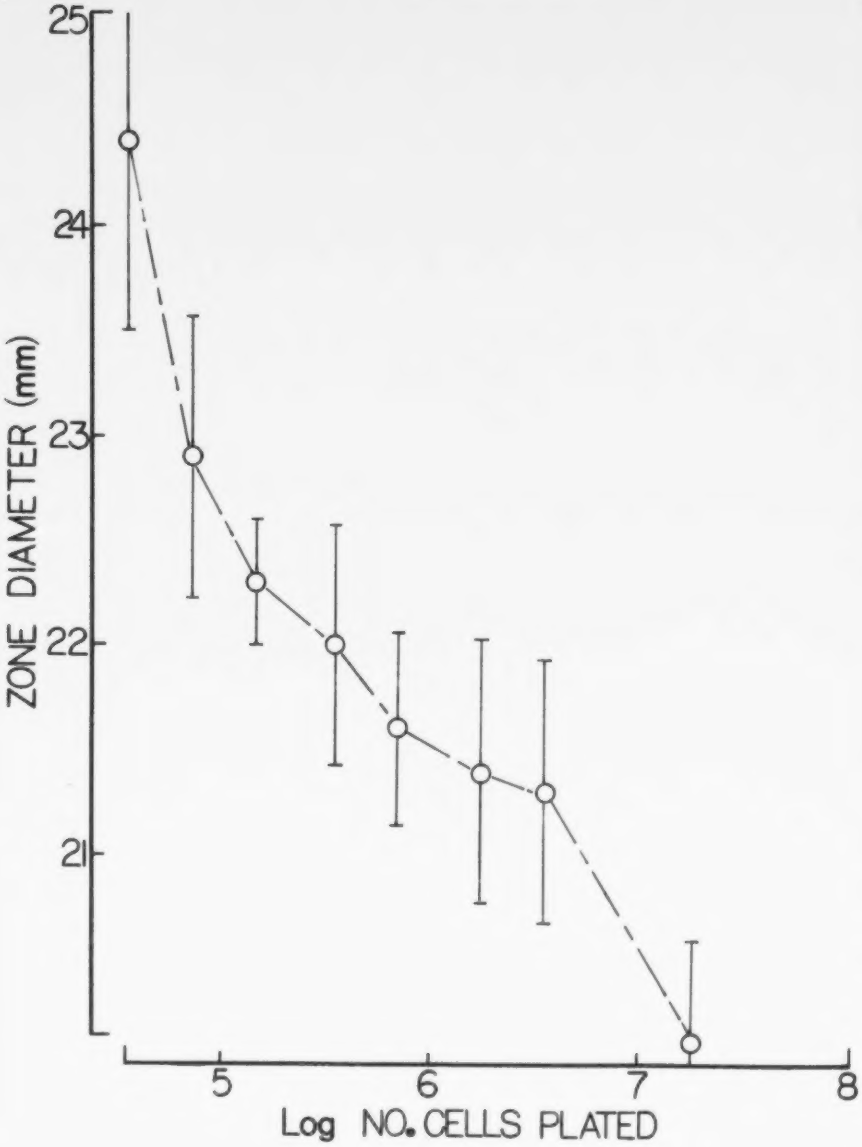

Figure 39. Inhibiton Zone Diameter as a Function of *E. coli* Cell Concentration

Inhibition zone diameter as a function of the log of the total number of *E. coli* (strain #40) cells plated. Each data point represents N = 9 replicates. Forty micrograms of sodium hypochlorite were applied to each disc.

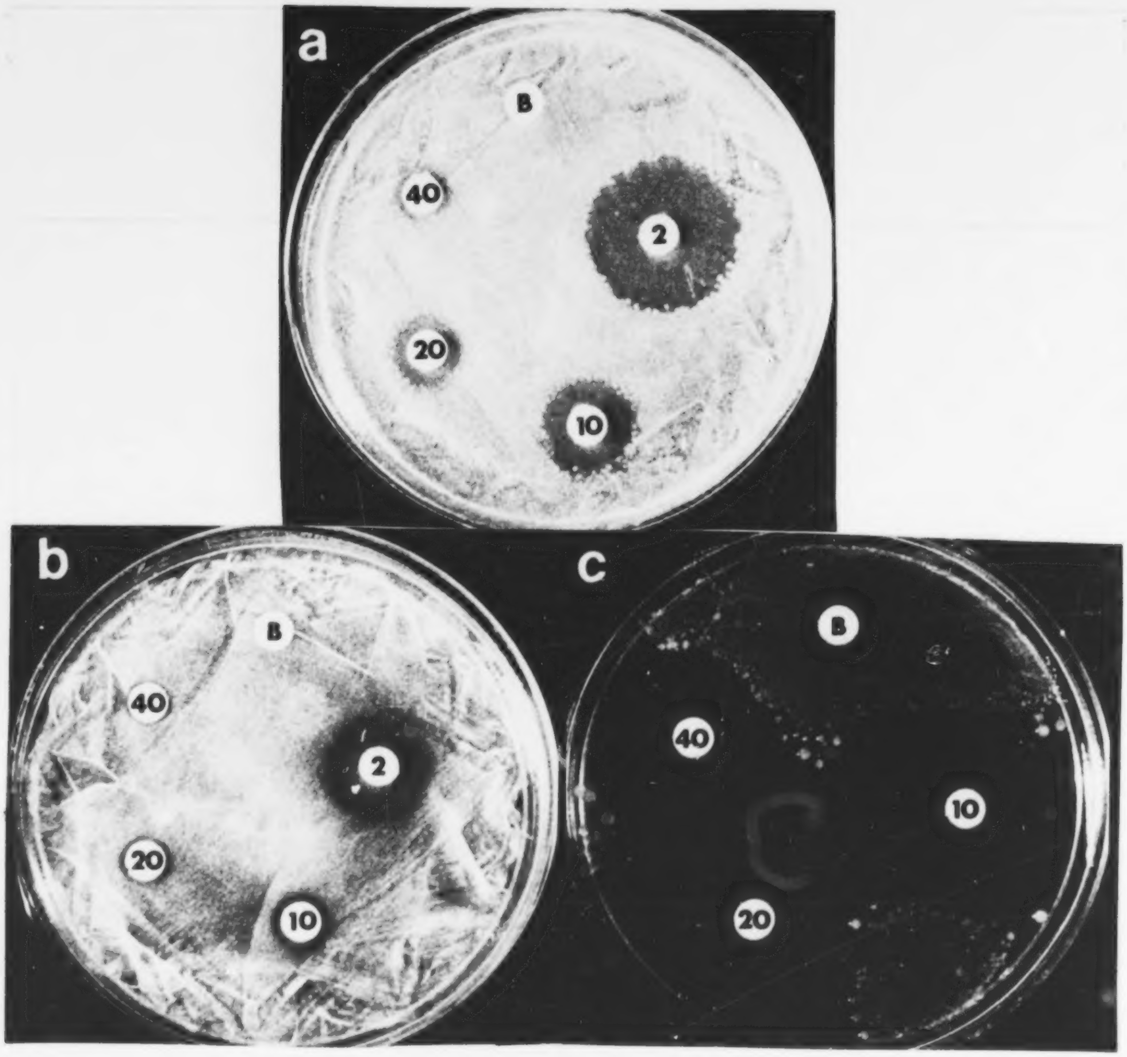

Figure 40. Comparison of Inhibition Zone Diameters for Three Bacterial Strains

Comparison of inhibition zone diameters on disc assay agar for three bacterial isolates. Plate *A* = *E. coli* (strain #40). Plate *B* = *Acinetobacter* sp. (strain IS, 1-2) from Irvine source water. Plate *C* = *Pseudomonas* sp. (strain 181 from Garden Grove system. Discs were saturated with: (B) = 8.0 μl of 10 mm potassium phosphate buffer, pH 7.0; (2) - 200 μg of buffered NaOCl; (10) = 40 μg of NaOCl; (20) = 20 μg of NaOCl; (40) = 10 μg of NaOCl. Order of sensitivity to combined chlorine = *Pseudomonas* sp.> *E. coli*> *Acinetobacter* sp.

sp. > E. coli (strain #40) > Acinetobacter sp. E. coli consistently yielded a zone diameter of 21.8 ± 1.19 mm (N = 100) when 40 micrograms of sodium hypochlorite was applied to the disc. A similar order of sensitivity was observed when the above three bacteria were tested for susceptibility to combined chlorine in a buffered liquid system (Figure 41), thereby confirming the ability of the disc technique to qualitatively estimate microbial tolerance to organically-bound chlorine.

As is the case in a liquid assay system, the bacteriocidal effect of the applied chlorine in the disc assay could be readily prevented by the addition of sodium thiosulfate to a proximal disc (Figure 42).

Application of the disc assay method. The disc assay technique was applied to a total of 215 randomly selected bacterial isolates from the Irvine and Garden Grove water distribution systems. In every instance the isolates from the unchlorinated Garden Grove system exhibited larger inhibition zones, indicating they were more susceptible to organically-combined chlorine. The Flavobacterium/Moraxella group exhibited the largest disparity in zone size between the two distribution systems. In the Irvine system, the Flavobacterium/Moraxella group showed the smallest zone diameter (18.7 ± 2.9 mm), making it the most resistant bacterial group, while the genus Pseudomonas was the most sensitive group (24.0 ± 2.0 mm). In the Garden Grove system, the "unknown" category of bacteria comprised the most resistant group (24.6 ± 4.6 mm), followed by Acinetobacter (29.1 ± 2.4 mm) and Klebsiella (29.1 ± 5.8 mm), respectively. The most sensitive group in the Garden Grove system was the Pseudomonas/Alcaligenes group (34.1 ±8.5 mm).

A frequency histogram summarizing the zone diameters for all 215 bacterial isolates examined from both water distribution systems is shown in Figure 43. The mean zone diameter for the Irvine bacterial isolates was 20.8 ± 3.26 mm (N = 130), while the mean zone diameter for the Garden Grove isolates was 30.1 ± 3.78 mm (N = 85). Though there was considerable overlap in the chlorine sensitivities of the individual isolates from the two systems, clearly the most sensitive bacteria originated from the Garden Grove system, while the most resistant microbial forms were from the Irvine system. Statistical analysis of these data using a standard t-test indicated that the Garden Grove isolates were significantly ($p \geq 0.001$) more susceptible to organically-combined chlorine than the Irvine isolates.

Standardization of the membrane filtration method. Nitrocellulose Gelman-type membrane filters, which are frequently employed in bacteriological work, were found to be unsuitable for use in the membrane filtration assay procedure, since they exhibited an excessively high chlorine demand (approximately 1.0 mg 1^{-1}). Instead, polycarbonate Nuclepore membrane filters, which had a very small, almost negligible chlorine demand, were utilized in the assay (Figure 44). The Nuclepore membranes possessed the additional advantages of a very uniform pore size and distribution, as well as an extraordinarily flat surface which enhanced contact between the bacterial cells and the overlying chlorine solution.

Using the membrane filtration technique, it was possible to measure the bacteriocidal effects of applied chlorine concentrations as low as 0.02 mg 1^{-1}

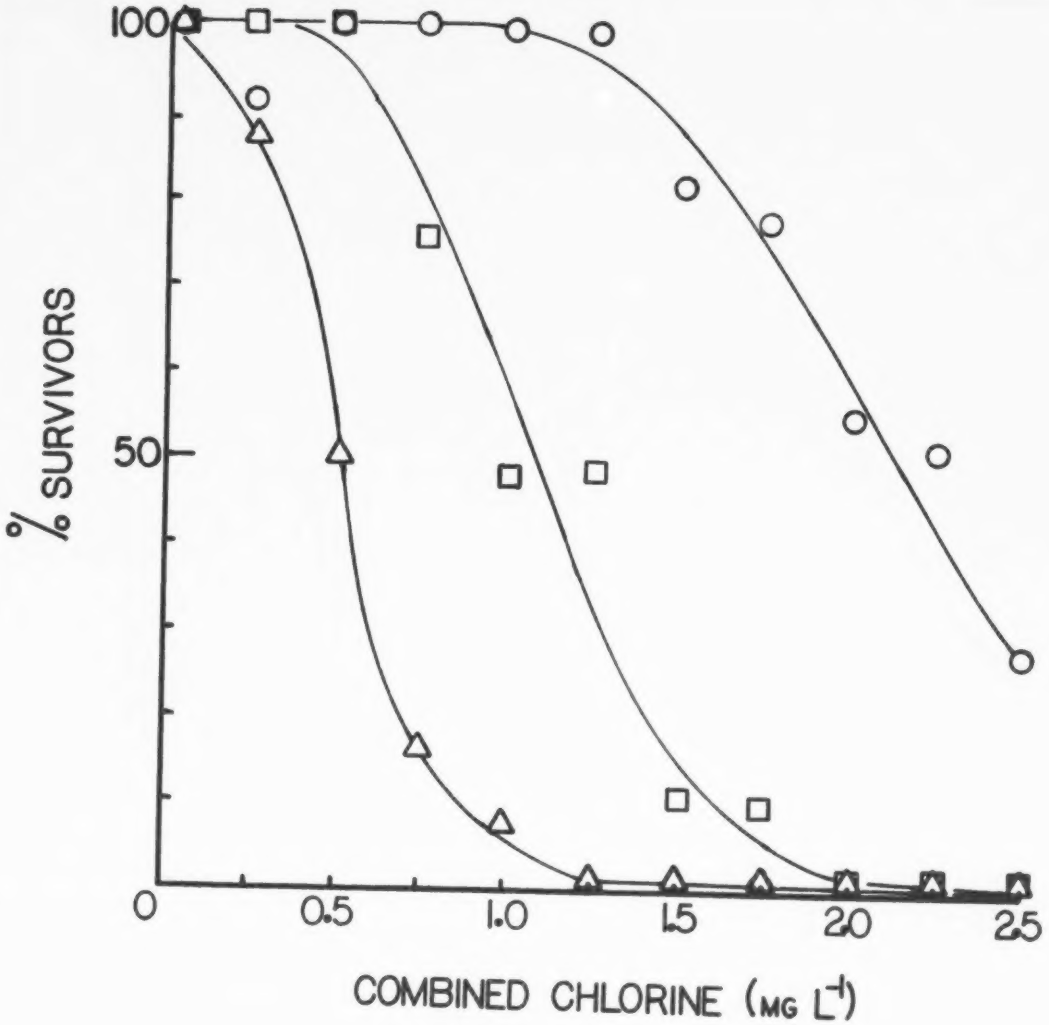

Figure 41. Bacterial Survival as a Function of Combined Chlorine Concentration in Phosphate-Buffered Liquid Suspension.

Bacterial survival as a function of combined chlorine concentration in a phosphate-buffered liquid suspension. Contact time = 30 minutes at 23°C and pH 7.0. Symbols: △ = Pseudomonas sp. (strain 181), □ = E. coli (strain #40), ○ = Acinetobacter sp. (strain IS, 1-2). Note that the relative order of sensitivity is the same as that shown in Figure 40.

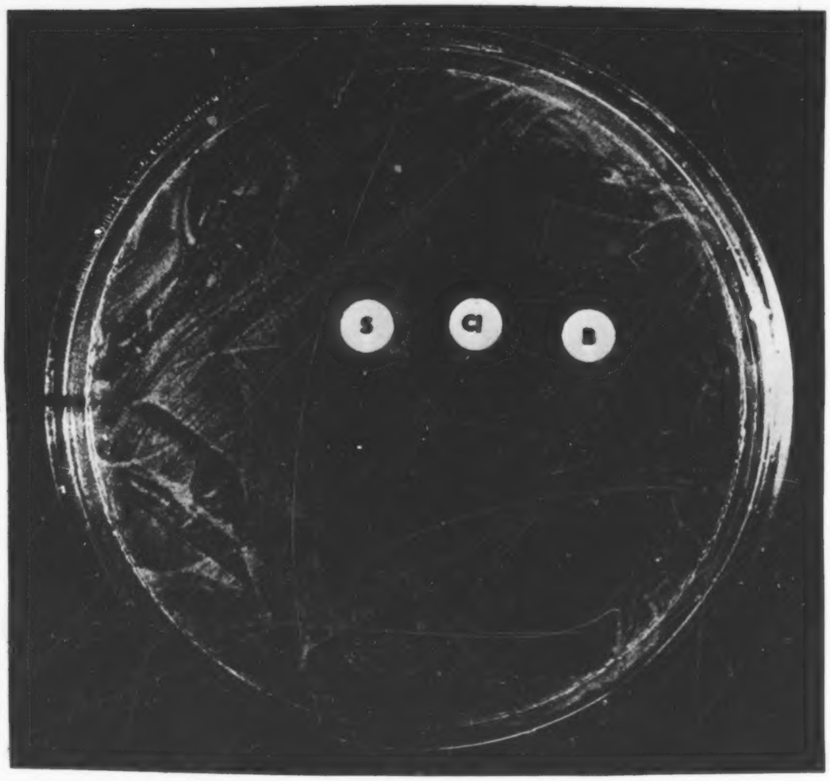

Figure 42. Effect of Sodium Thiosulfate in Neutralizing Combined Chlorine in the Disc Assay.

Control plate showing protection against combined chlorine toxicity by sodium thiosulfate in the disc assay procedure: (B) = 8.0 µl of 10.0 mM potassium phosphate buffer, pH 7.0 applied to disc; (Cl) = 200 µg of sodium hypochlorite applied to disc; (S) = 8.0 µl of 0.1 M sodium thiosulfate applied to disc. E. coli (strain #40) cells were used as the test organism.

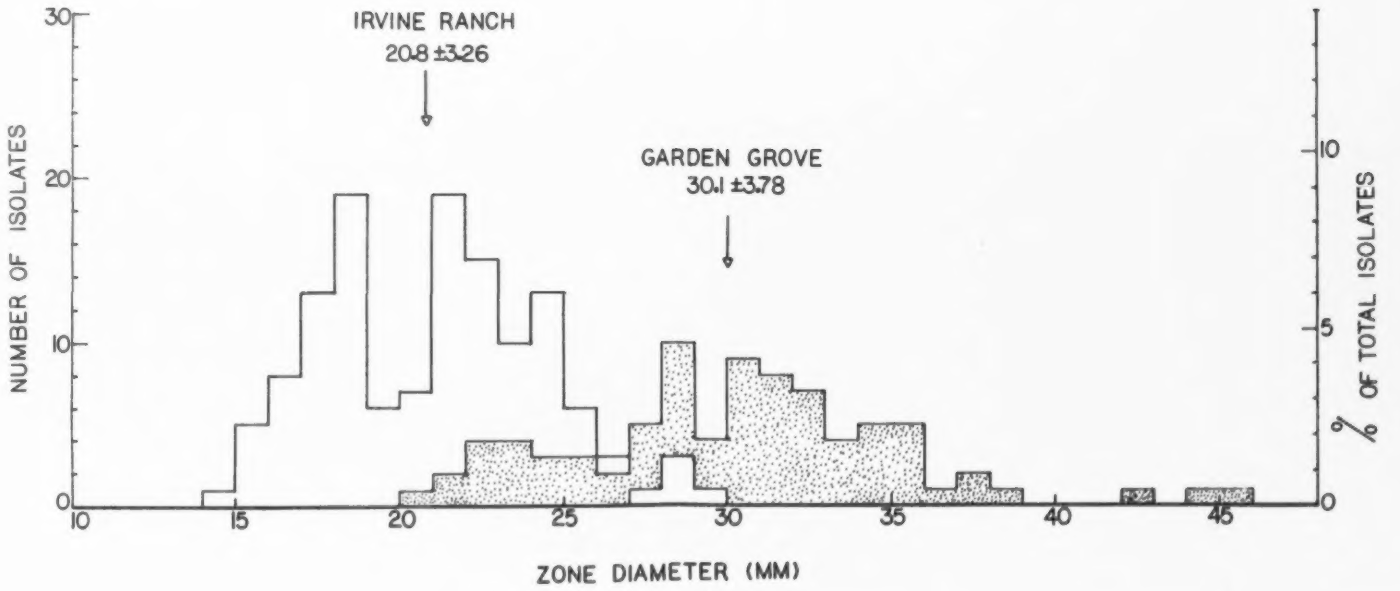

Figure 43. Comparison of Combined Chlorine Resistance of 215 Isolates from the Garden Grove and Irvine Water Distribution Systems

Frequency histogram showing inhibition zone diameters for 130 bacterial isolates from the Irvine system (open bars) and 85 isolates from the Garden Grove system (filled bars). The mean zone diameters and standard deviations for each group are given in mm.

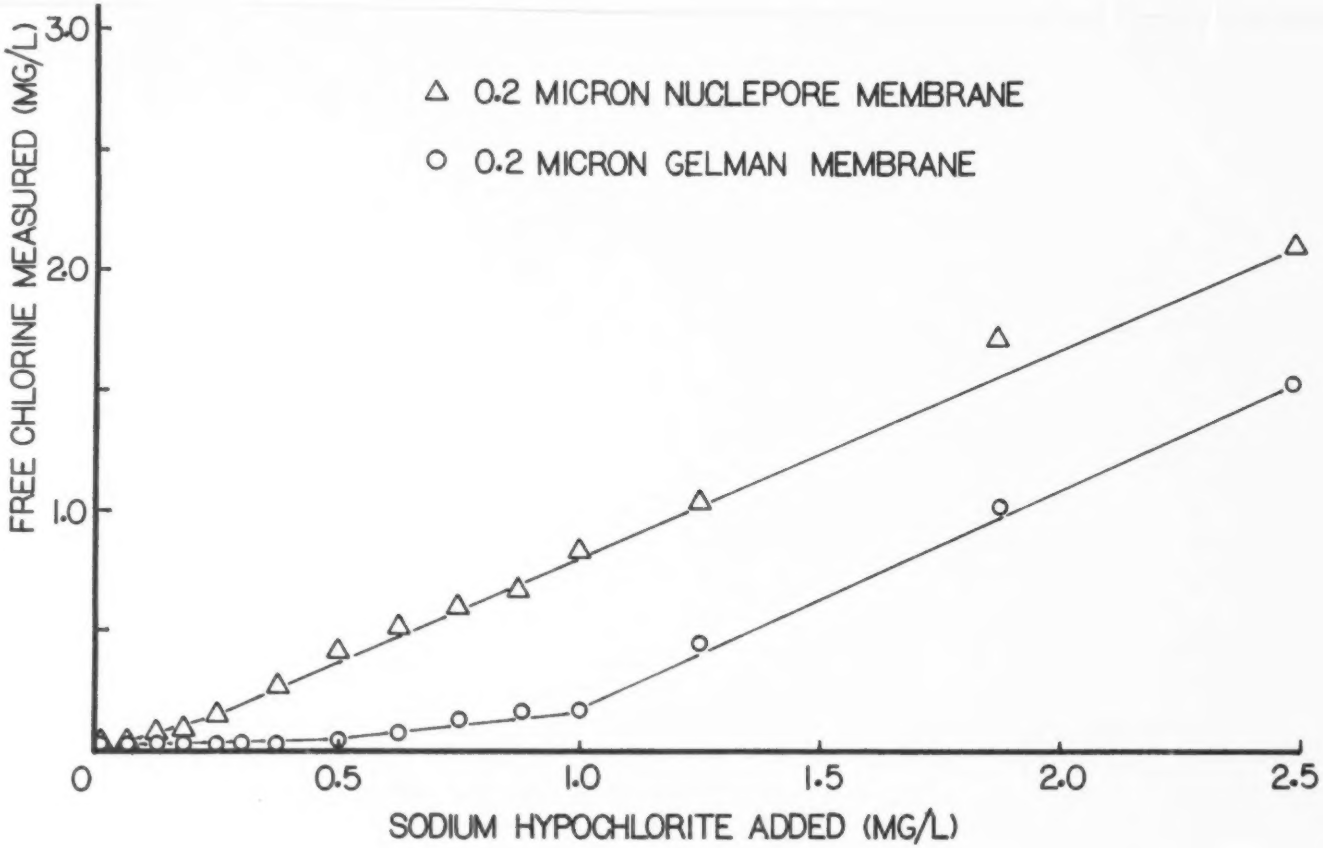

Figure 44. Chlorine Demand of Two Membrane Filters.

Concentration of free available chlorine (determined amperometrically) as a function of the applied chlorine concentration for polycarbonate Nuclepore membrane filters (0.2 μm pore size) and nitrocellulose Gelman type membrane filters (0.2 μm pore size). Contact time = 45 minures. Temperature = 23°C. pH = 7.0.

(Figure 45). In addition, the kinetics of disinfection on the Nuclepore membrane surface closely paralleled those in buffered liquid systems during the initial 10 to 20 seconds of exposure to chlorine (Figure 46). The rate of cell death on the membrane exhibited biphasic kinetics; during the initial 10 to 20 seconds, there was a two to three log decrease in cell viability, followed by a more gradual decline. Because the majority of bacterial die-off occurred so rapidly, a two-minute contact time was adopted for use in the standard assay. This amount of time was sufficient to attain near maximum kill at the various applied chlorine concentrations (see Materials and Methods).

Monitoring of the system amperometrically, or using the DPD procedure for detecting free chlorine, indicated that the free chlorine concentration overlying the membrane was essentially equivalent to the applied concentration throughout the two-minute contact period. Up to 2 x 10^9 bacteria could be filtered onto the Nuclepore membrane surface before a substantial decline was noticed in the amount of free available chlorine (Table 45). Using actual drinking water samples, the number of applied cells was always several orders of magnitude less than this. Thus under experimental conditions the contribution by the cells to the total chlorine demand was insignificant. In addition, the level of bacterial survival was evidently independent of the number of cells applied to the surface of the membrane filter, unless the numbers applied were sufficiently high to result in extensive clumping.

TABLE 45. EFFECT OF CELL NUMBER[1] ON CHLORINE DEMAND[2] IN MEMBRANE FILTRATION ASSAY PROCEDURE

Number of Cells Filtered	Free Chlorine Remaining (as mg l^{-1}) after: 0 min.	1 min.	2 min.
0	1.22	1.22	1.24
1.82 x 10^2	1.21	1.20	1.20
1.82 x 10^4	1.20	1.21	1.23
1.82 x 10^6	1.22	1.21	1.21
1.82 x 10^9	1.22	0.95	0.79

[1] Cells were E. coli (strain #40) grown overnight at 35°C in a minimal salts glucose broth.

[2] Free chlorine was determined by the DPD procedure (see Materials and Methods).

Application of membrane filtration method. Data from six separate experiments comparing the relative susceptibilities to free chlorine of bacteria recovered from the Irvine and Garden Grove systems are summarized in Figure

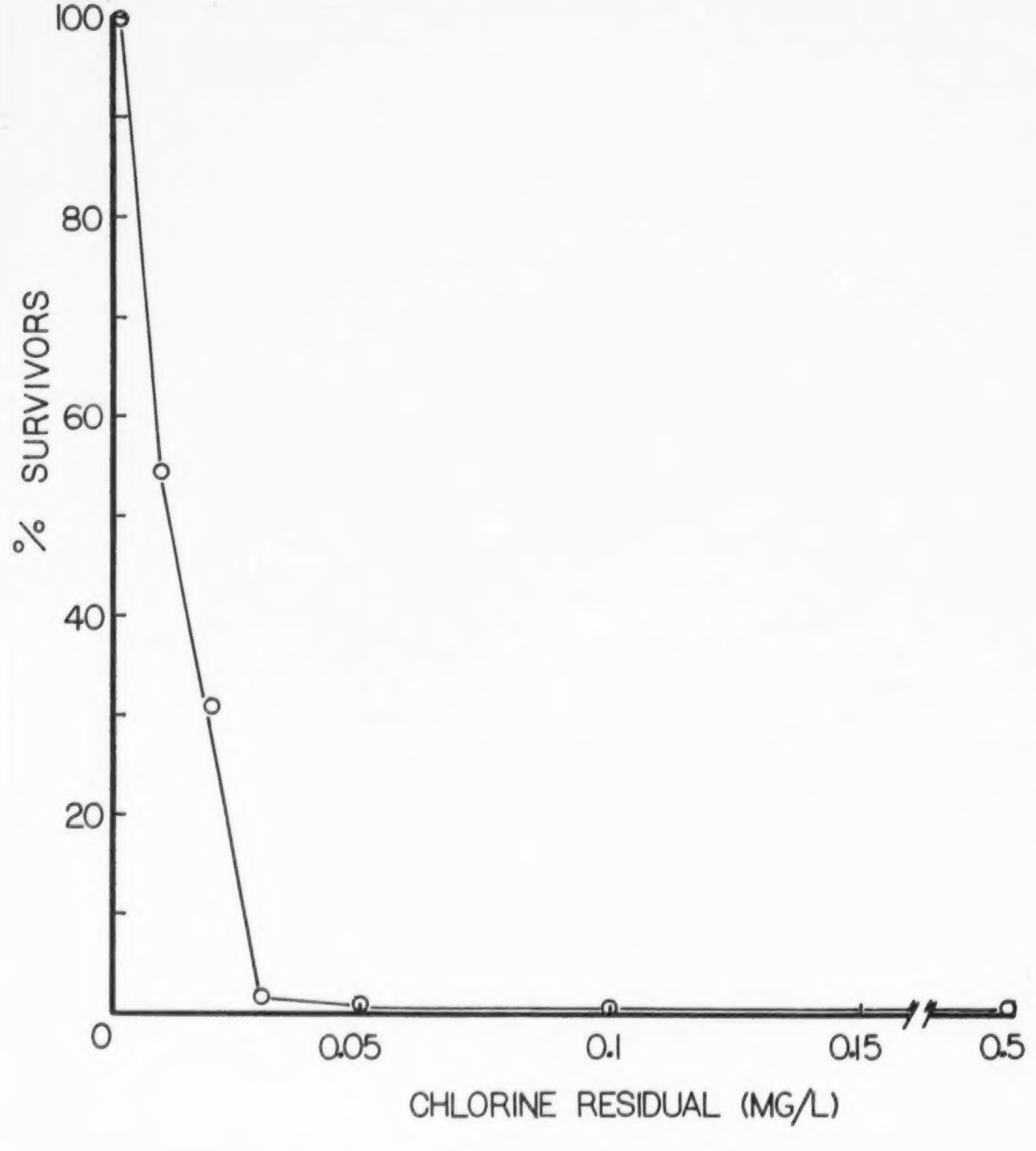

Figure 45. Survival of E. coli on 0.2 μm Nuclepore Membrane Filters.

Survival of E. coli (strain #40) on 0.2 μm pore-size Nuclepore membrane filters as a function of the applied chlorine concentration. Survival is expressed as a percentage of the total number of colonies appearing on untreated control membranes. Contact time = 2.0 minutes. Temperature = 23°C. pH = 7.0.

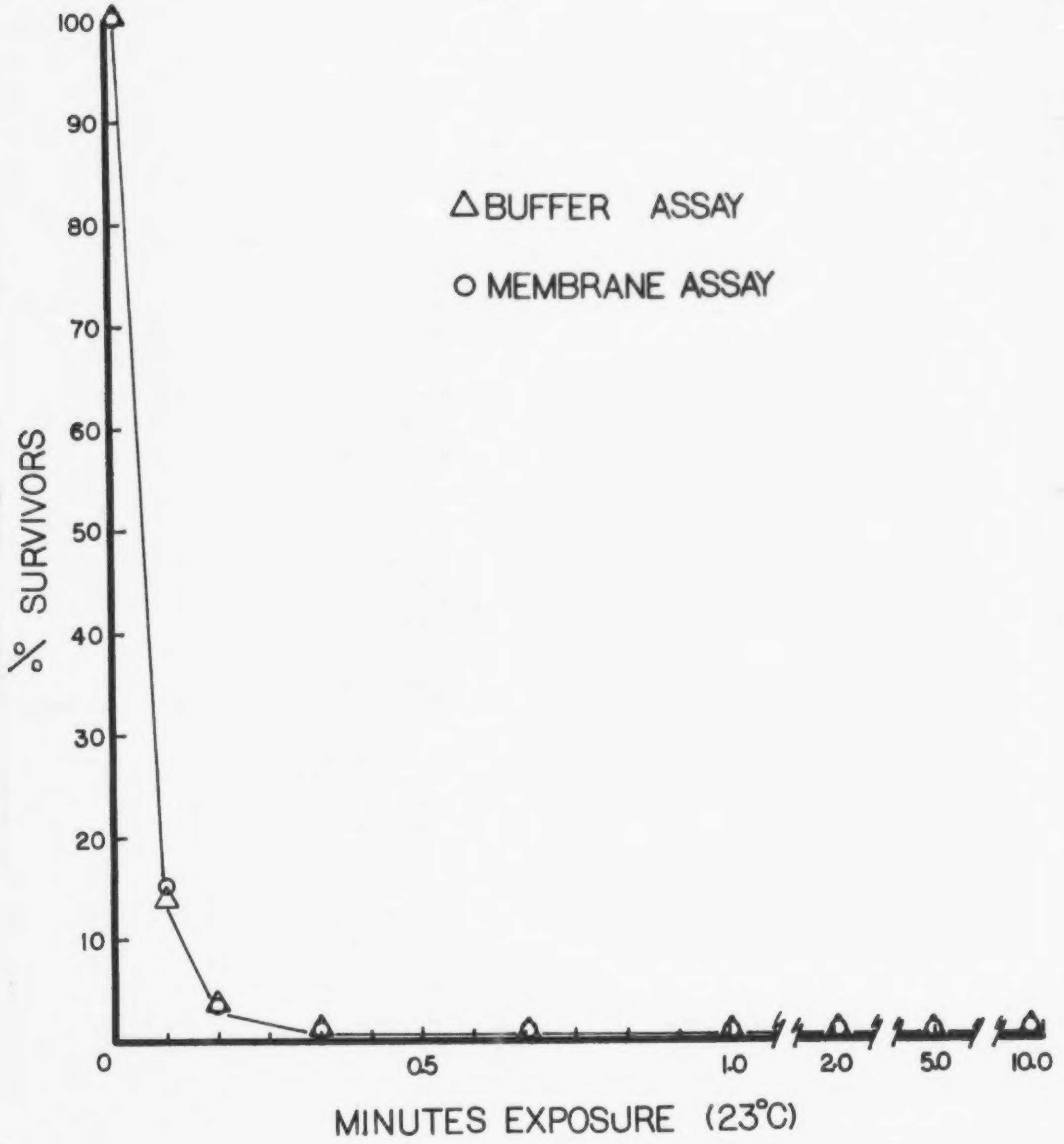

Figure 46. Kinetic of Chlorine Induced Cell Death of E. coli Using the Membrane Filter Assay Technique.

Kinetics of cell death of E. coli on 0.2 μm pore-size Nuclepore membrane filters (0) and in 10.0 mM potassium phosphate buffer, pH 7.0 (△). The initial free chlorine concentration in both the membrane and buffer assay was 0.1 mg l^{-1}. Temperature = 23°C. The reactions were stopped by the addition of 10.0 mM sodium thiosulfate and 0.1% peptone.

47. The Irvine bacteria were found to be significantly more resistant to chlorine disinfection at all concentrations of halogen tested between 0.1 and 10 mg l^{-1}. Although the Irvine bacteria were generally less numerous in situ than the Garden Grove bacteria (cf. Figure 1, 24), they exhibited up to 80-fold better survival when exposed to a 0.1 mg l^{-1} solution of sodium hypochlorite. This difference in sensitivity decreased at higher chlorine residuals and was virtually absent at a concentration of 100 mg l^{-1}. Approximately 0.5 percent of the total bacteria encountered in both distribution systems were highly resistant to chlorine. These bacteria were able to survive an exposure of 10 mg l^{-1} applied sodium hypochlorite for the duration of the two-minute contact period.

In order to determine which bacterial genera were most sensitive or resistant to chlorine disinfection, a total of 260 bacterial isolates from one experiment were identified following exposure to various concentrations of free chlorine in the membrane assay. The results, which are shown in Table 46, indicated that different microbial genera were killed by different levels of chlorination in the membrane filtration procedure. In the Garden Grove system, for example, the following groups of bacteria were killed at applied halogen concentrations of less than 0.5 mg l^{-1}: *Acinetobacter*, *Flavobacterium/Moraxella*, *Pseudomonas/Alcaligenes*, *Klebsiella*, *Micrococcus*, and *Corynebacterium/Arthrobacter*. Gram-positive spore-forming bacilli and actinomycetes were clearly the most chlorine-resistant cell types, comprising 48.3% and 51.7%, respectively, of the survivors following exposure to 10 mg l^{-1} free chlorine for two minutes. In the Irvine system, bacteria unable to survive free chlorine concentrations greater than 0.5 mg l^{-1} included *Acinetobacter*, *Pseudomonas/ Alcaligenes*, *Klebsiella*, and most of the Gram-positive micrococci. *Bacillus* sp. were the predominant chlorine-resistant forms in the Irvine system.

Mechanism of chlorine resistance. One mechanism for bacterial resistance to chlorination is cellular aggregation or adhesion to suspended particulate matter. In order to evaluate the significance of this survival mechanism in potable water systems, the membrane filtration assay was carried out using 0.2 µm and 2.0 µm pore-size Nuclepore membranes. Scanning electron microscopy performed in this laboratory, as well as differential size filtration experiments done by others using radiolabled cells (5), demonstrated that single bacteria in environmental samples are usually less than 1.0 µm in diameter and readily pass through Nuclepore membrane filters having pore diameters greater than 2.0 µm. Bacteria which are aggregated together or attached to the surfaces of suspended particulate matter should be preferentially retained by larger pore-size filters; hence, a greater proportion of these cells may survive exposure to chlorine.

The results of four independent experiments involving differential size filtration of bacteria from the Garden Grove system are shown in Table 47. At a concentration of 10 mg l^{-1} applied chlorine bacteria retained on the 2.0 µm membrane filters exhibited at least ten-fold better survival than did the total microbial population recovered on the 0.2 µm filters. In each of the above cases, the number of colonies arising on the chlorine-treated membrane were compared with the number appearing on an untreated control filter of the same pore size. In Figure 48 are shown similar data for one Garden Grove

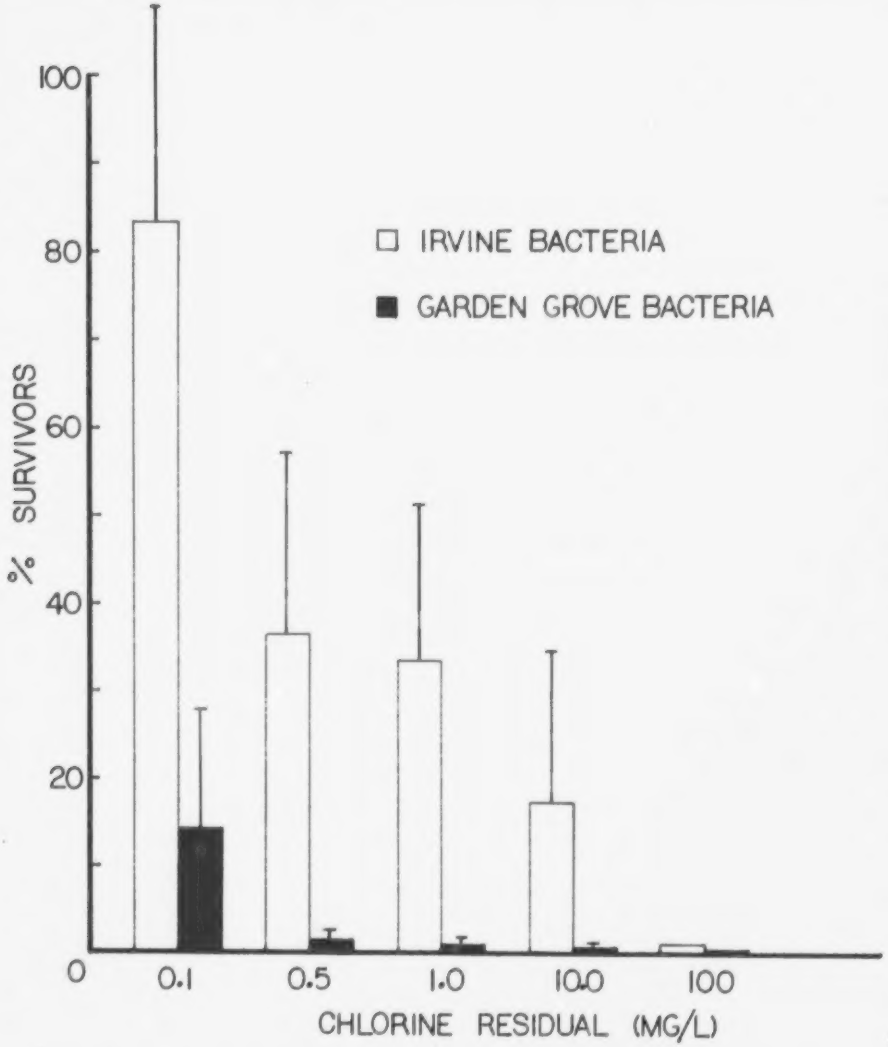

Figure 47. Survival of Irvine and Garden Grove Bacteria in the Chlorine-Membrane Filtration Test.

Survival of Irvine and Garden Grove bacteria as a function of applied chlorine concentration using the Nuclepore membrane filtration procedure. Data shown represent mean values from six independent experiments. Irvine water samples were obtained from the source hydrant location and Paseo Picasso hydrant site. Garden Grove samples were obtained from the Harris and Rockinghorse hydrant locations. Sampling dates: 9/12/79, 9/25/79, 9/27/79, 10/12/79, 10/23/79, 1/19/79. Average *in situ* concentration of free available chlorine for the Irvine samples: 0.82 ± 0.20 mg l^{-1}; Garden Grove samples: 0.017 ± 0.04 mg l^{-1}. Contact time = 2.0 minutes. Temperature - 23^{o}C. pH = 7.0.

TABLE 46. PERCENTAGES[1] OF BACTERIA SURVIVING EXPOSURE TO FREE CHLORINE IN MEMBRANE FILTRATION ASSAY PROCEDURE

Bacterial Genus/Taxonomic Group	Chlorine Concentration ($mg\ l^{-1}$)					
	0	0.1	0.5	1.0	10.0	
Acinetobacter	21.9	36.4	(14.3)	3.3		
Flavobacterium/ Moraxella		2.3				
Corynebacteria/ Arthrobacter	56.1	36.4	10.0			
Pseudomonas/ Alcaligenes	17.1 (3.0)	10.7				
Klebsiella		2.3 (3.6)				
Micrococcus	2.4 (84.8)	4.5				
Bacillus	(12.1)	4.5 (10.7)	2.3 (85.7)	40.0 (100.0)	48.3 (50.0)	
Actinomycete	2.4	13.6	66.7	56.7	51.7	
Unknown		(3.6)				
Number of Bacteria Identified	41 (33)	44 (28)	30 (7)	30 (14)	29 (4)	TOTAL: 29 (4)

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[1] Numbers in parentheses indicate Irvine data; other numbers indicate Garden Grove data. Irvine water sample was from Paseo Picasso hydrant location. Garden Grove water sample was from Harris hydrant location. Sampling date 1/19/81.

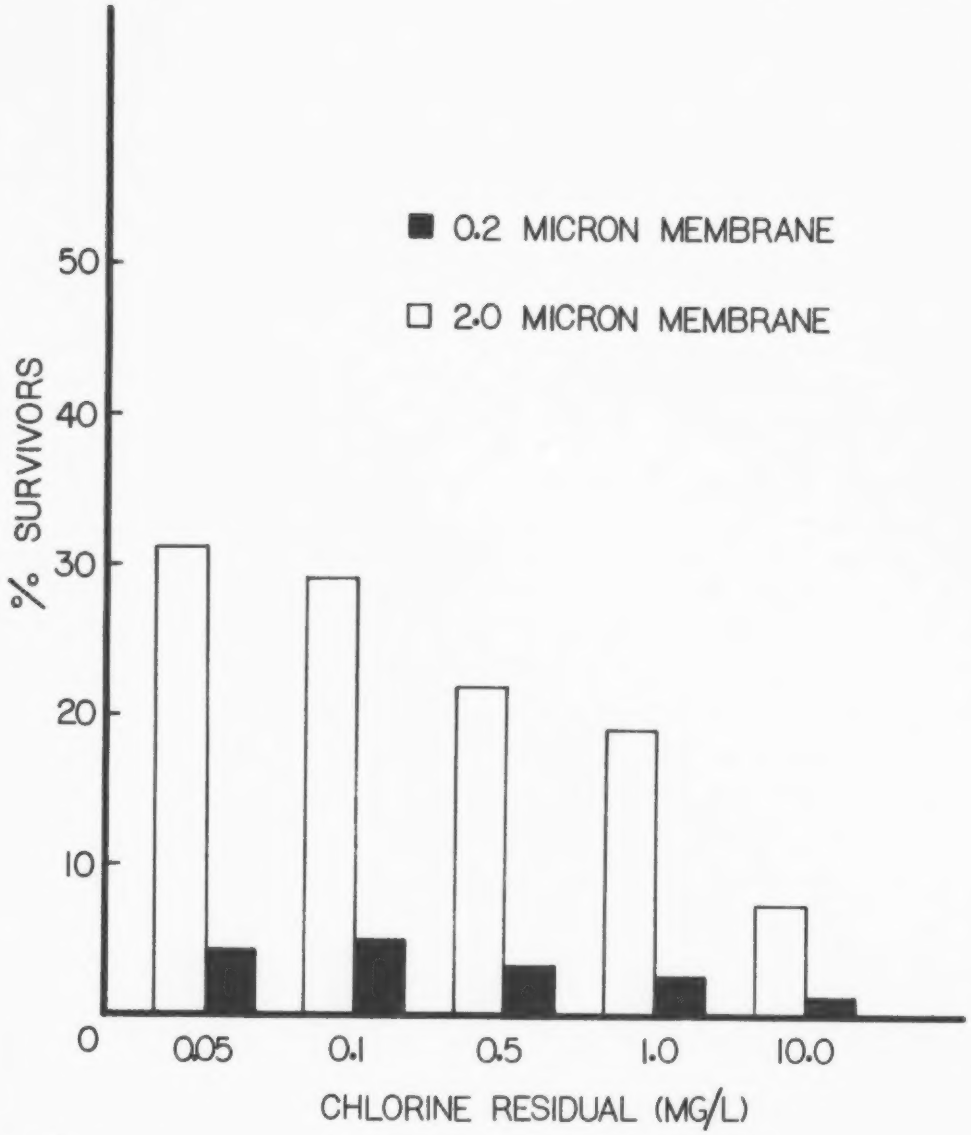

Figure 48. Comparison of Bacterial Survival to Free Residual Chlorine Using Two Different Pore-Sized Membranes.

Survival of bacteria from Garden Grove system (Harris Street) on 0.2 μm and 2.0 μm pore-size Nuclepore membranes as a function of the concentration of applied chlorine. Contact time = 2.0 minutes. Temperature = 23°C. pH = 7.0

water sample which cover a range of applied chlorine concentrations (0.05 to 10 mg l^{-1}). At all concentrations of halogen tested, the bacteria retained on the 2.0 μm filters showed significantly greater survival than those on the 0.2 μm filters, suggesting that unassociated bacteria are more sensitive than aggregated or attached cells. In addition, there was an obvious enrichment in the number of actinomycete colonies on the 2.0 μm control and chlorine-treated membrane filters, compared to the 0.2 μm filters. Of the bacteria which grew on the treated 2.0 μm membranes, from 30 to 60% corresponded to actinomycete colonies. Presumably, this enrichment was due to two factors: (i) the filamentous nature of actinomycetes, which would tend to impede their passage through a 2.0 μm membrane filter, and (ii) the formation of chlorine-resistant actinospores (62).

TABLE 47. BACTERIAL SURVIVAL ON 0.2 μm AND 2.0 μm NUCLEPORE MEMBRANE FILTERS*

	% Survival on:		
Membrane Pore Size	Control (No Treatment)	10 mg l^{-1} NaOCl (2.0 min. contact time)	
0.2	100	0.74	0.71
2.0	100	9.90	3.93

*Means and standard deviation from four experiments.

Harris St. (Garden Grove) water sample. Sampling dates: 4/2/80, 4/8/80, 4/18/80, 1/19/80.

Scanning electron microscopy. The higher chlorine resistance of the bacteria retained on the 2.0 μm pore-size Nuclepore membrane filters prompted a microscopic search for aggregated or attached cells. Scanning electron microscopy of suspended particulate matter recovered from the same water samples as used in the experiments described above revealed bacterially-colonized particles, as well as evidence of cellular aggregation (Figure 49). Approximately one percent of the particles examined bore attached bacterial cells, which were generally rod shaped, and sometimes partially obstructed from view by extracellular slime or capsular material. The number of bacteria affixed to a single particle varied from as few as five or ten, to as many as several hundred. Particles having attached bacteria were usually larger than about 10 μm, so they would almost certainly be captured on the 2.0 μm membrane filters. In addition to the colonized particles, branching filamentous microorganisms identical in morphology to actinomycetes were also frequently observed on the membrane filter surfaces. Although a higher resistance to chlorine disinfection was not directly demonstrated for the attached cells and actinomycete-like filaments observed in the scanning electron microscope,

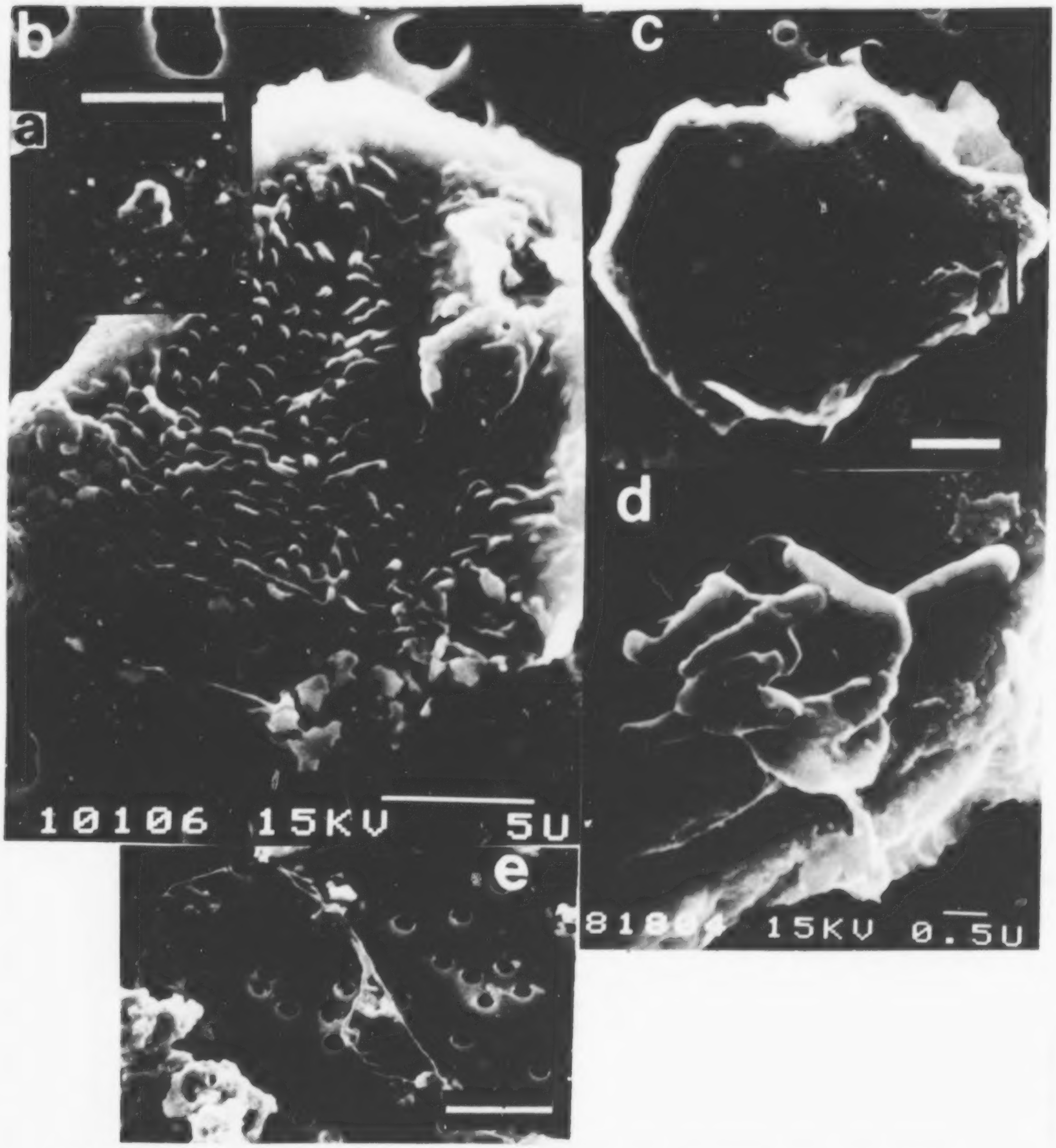

Figure 49. Scanning Electron Micrographs of Bacterially Colonized Particles

Scanning electron micrographs of bacteria-laden suspended particulate matter and Actinomycete-like filaments from the Garden Grove System. (A) = low magnification view of particles captured on the surface of 2.0 μm pore-size Nuclepore membrane (Bar = μm); (B) = enlargement of particle delineated in (D) showing attached bacterial cells partially coated by extra-cellular slime (Bar = 5.0 μm and 0.5 μm, respectively); (E) branching Actinomycete-like filaments (Bar = 10 μm their retention on the surfaces of 2.0 μm membrane filters lends support to this hypothesis.

Conventional kinetic methods for measuring microbial disinfection rates of free or combined chlorine are both labor intensive and time consuming, and therefore cannot be readily adapted to screening large numbers of microbial isolates. In addition, these methods generally involve subculturing of the bacterial isolates on artificial growth media followed by extensive washing of the cells prior to determination of disinfection kinetics, processes which inevitably lead to alteration of the cell surface characteristics and dramatic departure from actual *in situ* environmental conditions. Using the membrane filtration procedure described in this paper, it is possible to rapidly and quantitatively assess the chlorine resistance patterns of a mixed bacterial population obtained directly from the environment soon after sampling, as opposed to testing individual bacterial isolates after they have been repeatedly subcultured on artificial growth media. Possible physiological or genetic alteration of the bacteria which might result from subculturing and prolonged cold storage are thereby precluded. Dissolved organic-nitrogen compounds, free ammonia, or other dissolved contaminating substances in the water supply which may introduce extraneous chlorine demand can be readily eliminated from the membrane assay by very briefly rinsing the cells on the membrane surface immediately prior to chlorine exposure. The membrane procedure also facilitates the detection and axenic isolation of chlorine-resistant or sensitive cell types in the water sample. These bacteria can then be utilized in subsequent studies designed to elucidate the biochemical and physiological basis of extreme sensitivity or resistance to chlorine.

The disc assay method can also be performed rapidly and inexpensively, though it cannot be easily quantified and measures only the relative toxicity of combined chlorine. In addition, growth of the individual bacteria on artificial media is required. The chemical identities and concentrations of the organo-chlorine species formed as a result of diffusion of the chlorine from the filter paper disc into the growth medium were not investigated. However, the breakpoint chlorination characteristics of the disc assay broth indicated that all of the applied chlorine which diffuses from the disc must rapidly react with ammonia and organic-nitrogen compounds in the medium (95). The large chlorine demand displayed by the medium is due principally to amino acids, short-chain polypeptides, nucleic acids and proteins which consitute the tryptone and yeast extract components. The glucose, as well as the agar in the solid medium, would also exert a significant chlorine demand. These substances are present in the medium in a large molar excess compared to the amount of chlorine applied to the discs and are known to be extremely reactive with aqueous hypochlorous acid at neutral pH values (95, 97). Therefore, a wide variety of halogen-substituted organic compounds would be expected. Presumably, such compounds diffuse outward from the vicinity of the disc, establishing a concentration gradient; eventually, at some distance from the disc, a minimum inhibitory concentration would be attained which is characteristic for each bacterial isolate. Thus, the disc assay technique represents a qualitative determination of the relative susceptibility of individual bacterial isolates to a complex toxic mixture of chloramines and diffusible halogenated organic compounds.

The fact that relatively high numbers of bacteria could be regularly isolated from the Irvine system water containing in excess of 0.5 mg l^{-1} free chlorine suggests the possibility that certain bacteria may possess mechanisms which enable them to survive in highly chlorinated environments. A number of other conclusions may be inferred from the data obtained by the application of the disc assay and membrane filtration procedures, as described in this report. These conclusions are as follows: (i) Bacteria residing in the chlorinated Irvine water distribution system were, as a group, significantly more resistant to both the free and organically-combined forms of chlorine than bacteria which were found in the unchlorinated Garden Grove system. This suggests that there may be strong selection for chlorine-tolerant microorganisms in chlorinated water distribution systems; (ii) Bacteria within either distribution system showed marked individual differences in their sensitivities to disinfection by organically-combined chlorine in the disc assay; (iii) Results from the membrane filtration procedure indicated that gram-positive spore-forming bacilli and some micrococci were the predominant chlorine-resistant microbial genera detected in the chlorinated Irvine distribution system, whereas spore-forming bacilli and actinomycetes were the most resistant forms in the unchlorinated Garden Grove system; (iv) Filamentous bacteria such as actinomycetes, as well as bacteria attached to suspended particulate matter, or otherwise aggregated together in clumps which are too large to pass through a 2.0 μm filter, were apparently more resistant to disinfection by free chlorine than unassociated microorganisms.

Despite their apparent resistance to disinfection by free chlorine in the membrane filter assay, actinomycetes were generally not present in high numbers in the chlorinated Irvine system (83). However, LeChevallier et al. (73) recently demonstrated that actinomycetes were among the most frequently encountered groups of microorganisms in a chlorinated drinking water distribution system of an Oregon coastal community. Streptomycete spores have been shown to exhibit increased resistance to chloramines (62), which may account for their relatively high numbers in certain chlorinated waters. It is possible that apart from chlorination, other physico-chemical characteristics of the water supply, such as redox potential, dissolved oxygen, nitrate concentration, etc., may play an influential role in the proliferation of actinomycetes in one system and not in another. It was also shown by LeChevallier et al. (73) that certain gram-positive bacteria such as _Bacillus_ sp., _Micrococcus_ sp., and _Corynebacterium_ sp. in the chlorinated drinking water comprised nearly three times the number found in unchlorinated raw source water. In addition, the gram-negative genus _Acinetobacter_ showed a substantial increase compared to the total microbial population after experimentally exposing a raw water sample to 1.5 mg l^{-1} chlorine for one hour. However, the amount of free available chlorine was not stated in that study. According to the disc assay procedure, the genus _Acinetobacter_ was relatively resistant to combined chlorine, though both _Corynebacterium_ and _Acinetobacter_ were readily killed (in the membrane assay) by free chlorine concentrations of less than 0.5 mg l^{-1}. Interestingly, the _Acinetobacter_ isolates from the chlorinated Irvine system were relatively more resistant to combined chlorine (in the disc assay) than those from the Garden Grove system. Since a similar trend was noted for all of the other bacterial genera examined, different strains or species of the same genus may have the potential to develop increased resistance to combined-chlorine residuals.

The occurrence of bacterially-colonized suspended particulate matter in the Garden Grove water distribution system has been previously reported (106), and the chemical composition and numbers of these particles in water samples described (93). Though a higher chlorine resistance was not directly demonstrated for the attached bacteria as visualized in the scanning electron microscope, such bacteria-laden particles are evidently fairly common in raw water sources (42, 52, 80, 94) and certain municipal drinking water supplies (106). Their existance could account in part for the higher chlorine resistance of the bacteria retained on the 2.0 μm Nuclepore membrane filters. Microbial adhesion to solid surfaces is frequently mediated by extracellular acidic mucopolysaccharides (80), the manufacture and secretion of which by certain strains of *Ps. aeruginosa* has been shown to dramatically enhance resistance to disinfection by chlorine in swimming pool waters (111). It is possible that the extracellular material observed on the surfaces of the attached cells in this study may perform a similar protective function in chlorinated drinking water supplies.

Bacterial colonization of the surfaces of drinking water distribution pipes has been directly documented recently by the use of scanning electron microscopy (4, 106, 107). Further studies in this laboratory have demonatrated that such microbial colonization can occur even in highly chlorinated water systems (@ 1.0 mg/l) applied chlorine; unpublished observations). The cells comprising many of these microcolonies are frequently covered with extracellular substances, possible mucopolysaccharides, which may contribute to their ability to survive and multiply in such chlorinated environments.

Chlorination has been the most widely practiced method of disinfection for potable waters since the turn of the century, and the principle vehicle by which water quality is maintained. However, current public health standards for drinking water based on the coliform index fail to account for the sometimes high numbers of secondary opportunistic pathogens which these systems can harbor. In the environment, such bacteria apparently do not display a uniform sensitivity to free and combined chlorine, as suggested by the present study and other related investigations. Additional research will be required to more accurately assess the chlorine-resistance patterns of specific coliform and non-coliform bacterial populations as they exist in various raw and finished waters.

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APPENDIX A

CHEMICAL AND BACTERIAL PARAMETERS

TABLE A1. PHYSICAL AND CHEMICAL PARAMETERS FOR IRVINE SOURCE, PASEO PICASSO, AND L. PARKVIEW LANE

	OC-39 Source			Paseo Picasso			Parkview		
	$\overline{X}$[1]		S.D.	$\overline{X}$		S.D.	$\overline{X}$		S.D.
E.C. mhos/cm^2	894.0	±	50.0	89.0	±	72.0	918.0	±	29.0
TDS mg/l	527.0	±	31.0	521.0	±	34.0	488.0	±	52.2
pH units	7.8	±	0.4	7.7	±	0.6	7.75	±	0.54
D.O. mg/l	9.70	±	1.39	9.2	±	1.5	8.9	±	2.1
Turbidity (NTU)	0.42	±	0.19	2.3	±	7.4	2.83	±	8.38
				MAJOR CATIONS					
AMMONIA-N mg/l	0.023	±	0.037	0.026	±	0.036	0.033	±	0.060
SODIUM mg/l	86.6	±	3.8	85.7	±	3.3	84.8	±	2.3
POTASSIUM mg/l	4.9	±	0.28	4.9	±	0.3	4.9	±	0.3
CALCIUM mg/l	57.5	±	5.4	60.0	±	9.5	54.8	±	6.7
MAGNESIUM mg/l	25.0	±	2.4	23.3	±	7.1	26.7	±	2.0
				MAJOR ANIONS					
ALKALINITY mg/l	103.0	±	5.9	101.0	±	8.2	106.1	±	3.0
CHLORIDE mg/l	84.7	±	8.1	83.2	±	7.6	89.8	±	9.4
SULFATE mg/l	206.0	±	21.1	203.0	±	24.6	196.0	±	21.5
NITRATE mg/l	0.78	±	0.65	0.93	±	0.68	0.85	±	0.53
				TRACE ANALYSES					
	0.038	±	0.005	0.040	±	0.060	0.061	±	0.089
COBALT	0.0011	±	0.0009	0.001	±	0.001	0.0004	±	0.0005
COPPER	0.017	±	0.045	0.007	±	0.004	0.007	±	0.0035
IRON	0.027	±	0.019	0.122	±	0.122	0.087	±	0.094
MANGANESE	0.0035	±	0.0038	0.006	±	0.005	0.007	±	0.0035
ZINC	0.005	±	0.003	0.005	±	0.004	0.002	±	0.0008
TOC	2.70	±	0.95	3.06	±	1.25	2.97	±	0.59
PSD, Total	241.0	±	443.0	1463.0	±	1582.0	1225.0	±	735.0
Langlier Index	0.20	±	0.17	0.28	±	0.17	0.26	±	0.13
Chlorine Residual	0.73	±	0.14	0.60	±	0.15	0.60	±	0.20

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[1] Aithmetic Mean Value

TABLE A2. PHYSICAL AND CHEMICAL PARAMETERS FOR GARDEN GROVE SOURCE, ROCKINGHORSE, AND HARRIS

	Well 6321		Well 6064		Well 23/9		Rockinghorse		Harris	
	$\overline{X}^1$	S.D.	$\overline{X}$	S.D.	$\overline{X}$	S.D.	$\overline{X}$	S.D.	$\overline{X}$	S.D.
TDS mg/l	413.0	±15.0	368.0	±22.0	468.0	± 68.0	501.0	± 22.0	503.0	±106.0
E.C. mhos/cm^2	705.0	±27.0	648.0	±55.0	796.0	± 74.0	855.0	± 57.0	834.0	±157.0
pH units	7.2	± 0.6	7.4	± 0.5	6.9	± 0.5	7.4	± 0.4	7.4	± 0.5
D.O. mg/l	7.7	± 1.0	7.4	± 0.9	7.9	± 0.8	7.1	± 1.0	7.2	± 1.0
Turbidity	0.27	± 0.12	0.23	± 0.9	0.3	± 0.13	5.0	± 22.02	8.37	± 30.0
					MAJOR CATIONS					
AMMONIA-N	0.023	± 0.035	0.023	± 0.036	0.038	± 0.052	0.049	± 0.10	0.16	± 0.305
SODIUM	39.5	± 3.4	37.5	±	46.4	± 10.3	56.4	± 6.8	50.5	± 10.3
POTASSIUM	3.6	± 0.2	3.8	±	4.4	± 0.4	4.3	± 0.3	4.5	± 0.5
CALCIUM	84.3	± 3.3	74.1	±	90.1	± 10.7	85.9	± 15.1	98.0	± 19.5
MAGNESIUM	16.3	± 2.1	14.2	±	18.3	± 2.8	22.3	± 9.6	19.0	± 4.9
					MAJOR ANIONS					
ALKALINITY	180.0	± 5.3	174.0	± 9.4	178.0	± 7.9	179.0	± 22.9	173.0	± 5.8
CHLORIDE		±	41.5	± 4.9	58.2	± 11.1	67.6	± 5.9	65.1	± 18.6
SULFATE	96.3	± 7.3	79.8	±11.4	119.0	± 22.0	136.0	± 16.9	147.0	± 61.0
NITRATE as NO3	18.3	± 9.3	13.1	± 7.1	25.3	± 18.6	20.4	± 9.5	14.3	± 5.4
					TRACE ANALYSIS					
	0.046	± 0.060	0.039	± 0.049	0.036	± 0.053	0.046	± 0.062	0.037	± 0.065
COBALT mg/l	0.0012	± 0.0011	0.019	± 0.026	0.001	± 0.002	0.0017	± 0.0016	0.0008	± 0.00045
COPPER mg/l	0.016	± 0.020	0.113	± 0.263	0.150	± 0.114	0.008	± 0.008	0.017	± 0.0073
IRON	0.013	± 0.014	0.016	± 0.014	0.042	± 0.060	0.161	± 0.310	0.458	± 0.304
MANGANESE	0.045	± 0.005	0.003	± 0.038	0.003	± 0.002	0.007	± 0.008	0.016	± 0.0075
ZINC	0.020	± 0.016	0.064	± 0.136	0.056	± 0.096	0.008	± 0.009	0.0068	± 0.0036
TOC mg/l	0.50	± 0.23	0.53	± 0.38	0.5	± 0.15	1.0	± 0.83	0.54	± 0.13
PSD Total #/ml	30.5	±48.5	43.9	±68.4	304.0	±358.0	1090.0	±1025.0	2650.0	±809.0
Langlier Index	0.41	± 0.23	0.37	± 0.22	0.40	± 0.28	0.46	± 0.11	0.455	± 0.11
Chlorine Residual	0	±	0.001	± 0.005	0	±	0.04	± 0.06	0.014	± 0.051

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[1] Arithmetic Mean Value

TABLE A3. PARAMETERS MEASURED FROM JUNE 1978 THROUGH JUNE 1979
AT HARRIS STREET, GARDEN GROVE

	1978	MONTH				
Parameter	June	July	August	September	October	November
TPC35SP/ml	-	-	40.0±0.0	4850.0±1202.0	3205.0±4094.0	1422.5±173.2
TPCP35SP/ml	-	-	-	-	2420.0±3082.9	750.0±212.1
TPC35PP/ml	-	5000.0±0.0	-	-	-	250.0±0.0
TPCP35PP/ml	-	-	-	-	-	50.0±0.0
TC-MF-U/100ml	-	-	-	105.0±77.8	41.0±43.8	22.0±81.1
TC-MF-C/ml	-	-	-	-	-	44.0±0.0
M-FC/100ml	-	-	-	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.13±0.11	0.0±0.0	0.0±0.0
Temperature (^{o}C)	-	23.0±0.0	23.0±0.0	20.5±1.4	19.8±0.4	18.7±1.2
Dissolved Oxygen (ppm)	-	-	9.8±0.0	8.4±0.5	7.5±1.2	7.3±0.4
pH	-	8.4±0.0	7.5±0.0	6.8±0.0	-	6.6±0.9
E.C. (μmhos)	-	1000.0±0.0	1020.0±0.0	1172.0±0.0	1412.5±166.2	1332.5±24.8
Redox (mv)	-	-	190.0±0.0	-	-32.5±222.7	-36.0±161.2
Turbidity (NTU)	-	180.0±0.0	520.0±0.0	3.1±4.1	1.2±0.1	1.55±0.6

(continued)

TABLE A3 (continued)

Parameter	1978	1979	MONTH			
	December	January	February	March	April	May
TPC35SP/ml	1125.0±212.1	685.0±0.0	347.5±24.7	295.0±0.0	286.7±116.8	555.0±523.3
TPCP35SP/ml	622.5±187.9	350.0±0.0	208.0±45.2	202.5±109.6	153.3±101.5	512.5±548.0
TPC35PP/ml	612.5±165.2	785.0±0.0	468.0±87.7	512.5±17.7	420.0±173.9	310.0±183.8
TPCP35PP/ml	110.0±0.0	60.0±0.0	25.0±35.4	67.5±95.5	46.3±50.9	22.5±17.7
TC-MF-U/100ml	2.0±2.8	<1.0±0.0	1.5±2.1	2.0±1.4	3.0±5.2	<1.0±0.0
TC-MF-C/ml	<1.0±0.0	<1.0±0.0	0.5±0.7	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	17.3±0.5	17.0±0.0	16.8±1.1	17.5±0.7	19.3±1.1	19.7±0.2
Dissolved Oxygen (ppm)	6.3±1.9	6.7±0.0	7.3±0.4	7.6±0.0	-	6.6±0.2
pH	7.1±0.8	7.5±0.0	7.5±0.1	7.4±0.4	7.7±0.2	7.6±0.2
E.C. (μmhos)	1306.0±147.1	1400.0±0.0	1475.0±459.6	1375.0±601.0	940.0±96.4	875.0±91.9
Redox (mv)	75.0±35.4	0.0±0.0	12.5±102.5	110.0±282.8	130.0±52.9	107.5±45.9
Turbidity	1.1±0.0	0.2±0.0	0.4±0.3	0.4±0.2	0.6±0.5	0.5±0.1

(continued)

TABLE A3 (continued)

Parameter	1979 June	MONTH July	August	September	October	November
TPC35SP/ml	287.5±17.7	447.5±67.5	520.0±360.1	1035.0±1385.9	3310.0±643.5	9800.0±0.0
TPCP35SP/ml	117.5±17.7	110.0±155.5	331.7±372.6	37.5±10.6	5.0±0.1	0.0±0.0
TPC35PP/ml	315.0±254.6	282.5±38.8	397.5±392.4	2570.0±3577.9	2647.0±1035.9	2580.0±0.0
TPCP35PP/ml	12.5±3.5	0.0±0.0	50.0±70.7	12.5±17.6	45.0±7.0	0.0±0.0
TC-MF/U/100ml	0.5±0.7	4.5±4.9	<1.0±0.0	<1.0±0.0	<1.5±0.7	<1.0±0.0
TC-MF-C/ml	<1.0±0.0	2.5±2.1	<1.0±0.0	<1.0±0.0	<1.0±0.7	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100mL	<2.2±0.0	<2.3±0.0	6.8±7.9	9.1±9.7	<2.2±0.0	5.1±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	6.8±7.9	9.1±9.7	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.07±0.12	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	20.5±0.7	20.3±1.1	21.5±2.2	22.0±0.0	21.0±2.1	19.5±0.0
Dissolved Oxygen (ppm)	8.9±0.2	6.8±0.1	6.7±0.6	7.1±0.7	6.3±0.6	6.3±0.0
pH	7.5±0.0	7.8±0.4	7.4±0.2	7.5±0.14	7.4±0.2	7.1±0.0
E.C. (μmhos)	1000.0±141.4	767.5±243.9	1010.0±155.5	745.0±49.5	737.5±24.7	750.0±0.0
Redox (mv)	47.5±17.7	117.5±24.7	153.3±55.1	82.5±53.0	172.5±60.1	180.0±0.0
Turbidity (NTU)	2.5±1.8	2.8±3.1	4.8±3.7	5.4±0.6	4.95±0.78	5.2±0.0

(continued)

TABLE A3 (continued)

Parameter	1979 December	1980 January	MONTH February	March	April
TPC35SP/ml	880.0±0.0	2500.0±0.0	2060.0±0.0	166.0±0.0	950.0±0.0
TPCP35SP/ml	0.0±0.0	500.0±0.0	2060.0±0.0	0.0±0.0	0.0±0.0
TPC35PP/ml	705.0±0.0	1140.0±0.0	1735.0±0.0	108.0±0.0	990.0±0.0
TPCP35PP/100ml	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	5.1±0.0	5.1±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-
Temperature (°C)	15.5±0.0	17.0±0.0	17.5±0.0	18.0±0.0	19.0±0.0
Dissolved Oxygen (ppm)	6.7±0.0	7.2±0.0	7.4±0.0	7.2±0.0	6.5±0.0
pH	7.8±0.0	7.2±0.0	7.0±0.0	7.9±0.0	7.2±0.0
E.C. (μmhos)	760.0±0.0	520.00.0	550.0±0.0	375/0±0.0	660.0±0.0
Redox (mv)	-	190.0±0.0	180.0±0.0	135.0±0.0	80.0±0.0
Turbidity	2.6±0.0	2.6±0.0	2.6±0.0	1.2±0.0	1.4±0.0

TABLE A4. PARAMETERS MEASURED FROM JUNE 1978 THROUGH JUNE 1979
AT MARSHALL LANE, GARDEN GROVE

Parameter	1978		MONTH				
	June	July	August	September	October	November	December
TPC35SP/ml	-	-	20.0±0.0	872.5±321.7	158.0±0.0	880.0±975.8	145.0±120.2
TPCP35SP/ml	-	-	-	-	45.0±0.0	260.0±360.6	120.2±25.0
TPC35PP/ml	-	-	-	-	-	1050.0±0.0	85.0±0.0
TPCP35PP/ml	-	-	-	-	-	120.0±0.0	0.0±0.0
TC-MF-U/100ml	-	-	-	150.5±211.4	410.0±0.0	6.0±8.5	<1.0±0.0
TC-MF-C/100ml	-	-	-	-	-	<1.0±0.0	<1.0±0.0
M-FC/100ml	-	-	-	-	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.1±0.0	0.05±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	-	-	22.0±0.0	21.8±1.8	23.0±0.0	19.3±1.1	17.3±1.1
Dissolved Oxygen (ppm)	-	-	7.6±0.0	8.4±0.7	8.2±0.0	7.1±0.1	6.4±1.9
pH	-	-	11.0±0.0	6.4±0.2	-	7.6±0.1	6.8±0.4
E.C. (μmhos)	-	-	1460.0±0.0	994.0±0.0	1470.0±0.0	1372.0±110.3	1374.0±100.4
Redox (mv)	-	-	94.0±0.0	-	-225.0±0.0	-51.0±182.4	15.0±49.5
Turbidity (NTU)	-	-	5.6±0.0	1.9±1.5	53.0±0.0	36.6±40.2	6.0±1.4

(continued)

TABLE A4 (continued)

Parameter	1979 January	MONTH February	March	April	May	June
TPC35SP/ml	241.0±41.0	510.0±410.0	107.0±96.2	121.7±82.2	192.5±10.6	2682.5±3701.7
TPCP35SP/ml	127.0±159.8	62.5±3.5	7.0±9.9	0.0±0.0	15.0±21.2	900.0±1272.7
TPC35PP/ml	104.0±43.8	426.5±309.0	91.0±17.0	126.0±74.7	210.0±148.5	45.0±0.0
TPCP35PP/ml	4.0±1.4	29.0±1.4	0.0±0.0	3.3±5.8	15.0±21.2	0.0±0.0
TC-MF-U/100ml	2.0±2.8	6.0±2.8	<1.0±0.0	1.7±1.5	3.0±4.2	5.5±4.9
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	2.5±2.1
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<9.1±9.8
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	3.2±1.7	<2.2±0.0	10.55±7.7
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0+0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0+0.0
Temperature (°C)	16.5±0.7	16.8±1.1	17.8±1.1	19.5±0.7	20.8±1.8	20.3±0.4
Dissolved Oxygen (ppm)	5.8±1.1	6.5±1.9	8.6±0.0	-	6.7±0.0	6.7±0.9
pH	7.1±0.4	7.4±0.7	7.3±0.3	7.8±0.3	7.5±0.4	7.6±0.0
E.C. (μmhos)	955.0±1166.7	1485.0±445.5	1227.5±357.1	1116.7±228.5	750.0±56.6	875.0±120.2
Redox (mv)	43.5±132.2	25.0±91.9	202.3±194.5	46.7±80.4	107.5±53.0	10.0±98.9
Turbidity (NTU)	2.5±1.1	2.4±1.6	1.4±0.6	2.2±0.7	1.55±0.5	3.25±2.8

TABLE A5. PARAMETERS MEASURED FROM JUNE 1978 THROUGH JUNE 1979 AT ROCKINGHORSE, GARDEN GROVE

Parameter	MONTH June	July	August	September	October	November
TPC35SP/ml	-	1000.0±0.0	330.0±325.3	1436.5±1051.5	474.0±161.2	317.5±46.0
TPCP35SP/ml	-	-	-	-	172.5±222.7	51.5±65.8
TPC35PP/ml	-	-	-	-	-	600.0±0.0
TPCP35PP/ml	-	-	-	-	-	0.0±0.0
TC-MF-U/100ml	-	-	-	285.0±403.1	81.0±1.4	14.0±2.8
TC-MF-C/ml	-	-	-	-	-	<1.0±0.0
M-FC/100ml	-	-	-	-	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	-	5.7±4.9	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.08±0.04	0.04±0.02	0.0±0.0
Temperature (°C)	-	25.0±0.0	23.3±1.1	21.3±1.8	20.5±0.0	18.9±1.6
Dissolved Oxygen (ppm)	-	-	8.7±0.1	7.6±0.5	7.3±1.1	7.3±0.1
pH	-	8.2±0.0	7.7±0.1	6.8±0.4	-	7.2±0.9
E.C. (μmhos)		960.0±0.0	1210.0±14.1	1204.0±0.0	1471.0±1.4	1393.5±79.9
Redox (mv)	-	-	131.5±19.1	-	-5.0±176.8	37.5±53.0
Turbidity (NTU)	-	140.0±0.0	4.9±3.5	1.2±1.3	0.8±0.3	0.6±0.3

(continued)

TABLE A5 (Continued)

Parameter	1978 December	1979 January	February	March	April	May
TPC35SP/ml	682.5±130.8	547.5±109.6	188.0±73.5	1394.5±1733.1	322.0±298.6	242.5±123.7
TPC35SP/ml	57.5±67.2	132.5±95.5	36.0±22.6	1276.0±1801.7	66.7±73.2	50.0±28.3
TPC35PP/ml	420.0±162.0	541.0±203.6	287.5±31.8	205.0±35.3	350.0±173.8	280.0±84.8
TPCP35PP/ml	55.0±0.0	27.5±38.9	8.5±12.0	12.5±17.7	1.7±2.88	0.0±0.0
TC-MF-U/100ml	14.0±14.1	5.0±1.4	1.0±1.4	7.0±8.5	2.3±4.0	1.0±1.4
TC-MF-C/ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	0.5±0.7	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	3.7±2.1	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	3.7±2.1	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	3.7±2.1	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (C°)	17.0±0.7	16.3±0.4	16.5±0.0	17.8±1.1	19.8±1.1	21.3±0.4
Dissolved Oxygen (ppm)	6.2±2.2	6.1±1.9	7.2±0.6	7.5±0.0	-	5.9±0.0
pH	7.0±0.7	7.5±0.1	7.4±0.0	7.4±0.0	7.6±0.3	7.2±0.3
E.C. (μmhos)	1249.5±142.1	1285.0±586.9	1225.0±247.5	1270.0±381.8	1023.3±404.6	755.0±49.5
Redox (mv)	97.5±17.7	75.0±106.1	35.0±91.9	145.0±120.2	61.7±23.6	117.5±31.8
Turbidity (NTU)	0.7±0.3	0.3±0.07	0.4±0.1	0.4±0.1	0.6±0.6	0.4±0.0

(continued)

TABLE A5 (Continued)

Parameter	1979 June	July	August	September	October	November
TPC35SP/ml	252.5±208.6	156.0±189.5	80.0±77.8	942.5±611.6	799.0±977.2	85.0±0.0
TPCP35SP/ml	35.0±0.0	3.5±2.1	25.0±35.5	212.5±102.5	262.0±308.3	10.0±0.0
TPC35PP/ml	140.0±14.1	132.5±180.3	140.0±133.3	792.5±484.3	725.0±912.2	95.0±0.0
TPCP35PP/ml	5.0±7.1	0.0±0.0	16.7±28.8	155.0±7.1	37.0±10.6	0.0±0.0
TC-MF-U/100ml	44.5±57.3	<1.0±0.0	<1.0±0.0	<1.5±0.7	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	32.5±44.5	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC /100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	9.1±9.8	<2.2±0.0	<2.2±0.0	7.2±2.9	9.1±9.7	9.2±0.0
CONF-COL/100ml	16.0±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	9.1±9.7	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	.16±0.03	.14±0.06	.07±0.0	.05±0.07	0.0±0.0	0.0±0.0
Temperature (^{o}C)	21.8±1.1	22.5±0.5	23.3±0.0	23.8±0.6	22.2±0.0	20.0±0.0
Dissolved Oxygen (ppm)	7.8±1.4	7.6±1.1	7.5±0.8	6.6±0.3	7.4±1.4	6.5±0.0
pH	7.4±0.0	7.6±0.3	7.4±0.2	7.5±0.3	7.4±0.3	7.2±0.0
E.C. (mhos)	885.0±134.4	790.0±226.0	792.0±24.7	750.0±0.0	695.0±0.0	650.0±0.0
Redox (mv)	102.0±3.5	123.0±27.5	155.0±65.0	160.0±84.8	125.0±49.5	160.0±0.0
Turbidity (NTU)	5.6±7.1	3.4±5.0	2.1±0.6	3.8±4.3	1.0±0.42	0.5±0.0

(continued)

TABLE A5. (continued)

Parameter	1979 December	January	MONTH February	1980 March	April
TPC35SP/ml	175.0±0.0	122.0±0.0	275.0±0.0	277.0±0.0	550.0±0.0
TPCP35SP/ml	0.0±0.0	36.0±0.0	10.0±0.0	10.0±0.0	0.0±0.0
TPC35PP/ml	80.0±0.0	65.0±0.0	125.0±0.0	202.0±0.0	200.0±0.0
TPCP35PP/ml	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	9.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	18.0±0.0	17.0±0.0	18.0±0.0	18.5±0.0	19.0±0.0
Dissolved Oxygen (ppm)	7.4±0.0	6.4±0.0	6.8±0.0	6.5±0.0	6.0±0.0
pH	7.7±0.0	6.5±0.0	6.9±0.0	7.9±0.0	7.2±0.0
E.C. (μmhos)	610.0±0.0	590.0±0.0	635.0±0.0	530.0±0.0	525.0±0.0
Redox (mv)	-	140.0±0.0	160.0±0.0	145.0±0.0	60.0±0.0
Turbidity (NTU)	0.5±0.0	0.8±0.8	0.5±0.0	0.6±0.0	0.3±0.0

TABLE A6. PARAMETERS FROM JUNE 1978 THROUGH JUNE 1979 AT SANDRA #2, GARDEN GROVE

	1978	MONTH				
Parameter	June	July	August	September	October	November
TPC35SP/ml	-	-	270.0±325.3	832.5±7.8	187.5±109.6	170.0±120.2
TPCP35SP/ml	-	-	-	-	22.5±10.6	2.5±3.5
TPC35PP/ml	-	1000.0±0.0	-	-	-	280.0±0.0
TPCP35PP/ml	-	-	-	-	-	0.0±0.0
TC-MF-U/100ml	-	-	-	595.0±431.3	56.0±33.9	15.0±21.2
TC-MF-C/ml	-	-	-	-	-	<1.0±0.0
M-FC/100ml	-	-	-	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.03±0.04	0.03±0.04	0.0±0.0
Temperature (°C)	-	22.0±0.0	20.8±0.4	22.3±1.8	22.3±1.8	20.3±0.4
Dissolved Oxygen (ppm)	-	-	8.5±0.8	7.5±0.6	7.9±0.9	7.7±0.4
pH	-	8.2±0.0	7.1±1.1	6.0±0.0	-	7.2±0.3
E.C. (μmhos)	-	630.0±0.0	1000.0±169.7	1068.0±0.0	1481.0±12.7	1406.0±5.7
Redox (mv)	-	-	170.0±0.0	-	-23.5±214.3	11.0±86.3
Turbidity (NTU)	-	21.0±0.0	0.9±0.4	2.9±2.9	0.8±0.0	0.8±0.6

(continued)

Table A6. (continued)

Parameter	December	1979 January	February	March	April	May
TPC35SP/ml	442.5±512.7	197.5±180.3	176.0±43.8	115.0±22.6	116.7±33.3	267.5±265.2
TPCP35SP/ml	40.0±14.1	10.0±14.1	29.5±34.6	3.5±3.53	6.7±11.5	10.0±14.1
TPC35PP/ml	165.0±113.1	235.0±240.4	212.0±16.9	93.0±26.9	375.0±506.7	380.0±70.7
TPCP35PP/ml	0.0±0.0	2.5±3.5	6.5±9.2	1.0±1.4	1.7±2.9	12.5±10.6
TC-MF-U/100ml	2.0±2.8	5.0±7.1	<1.0±0.0	6.5±3.5	2.0±3.5	13.5±19.1
TC-MF-C/ml	<1.0±0.0	5.0±7.1	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	3.7±2.1	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	3.7±2.1	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	3.7±2.1	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	1.1±1.6	0.05±0.07	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	17.5±0.7	16.5±7.1	16.5±7.1	17.5±0.7	19.0±0.7	20.0±0.7
Dissolved Oxygen (ppm)	6.7±1.9	6.7±1.5	8.4±0.2	8.3±0.0	-	6.6±0.0
pH	6.9±0.6	7.2±0.0	7.5±0.1	7.2±0.2	7.5±0.1	7.5±0.1
E.C. (μmhos)	1186.0±128.7	1225.0±601.0	1355.0±487.5	1200±353.6	793.3±130.1	717.5±53.0
Redox (mv)	90.0±0.0	160.0±28.3	83.5±2.1	95.0±148.5	56.7±40.4	110.0±28.3
Turbidity (NTU)	0.7±0.1	0.2±0.0	0.2±0.0	0.4±0.2	0.3±0.1	1.1±0.5

(continued)

TABLE A6. (continued)

	1979		MONTH	
Parameter	June	July	August	September
TPC35SP/ml	382.5±321.7	2412.5±3039.6	488.3±428.8	270.0±176.8
TPCP35SP/ml	15.0±21.2	872.5±1070.2	151.7±100.5	42.5±38.9
TPC35PP/ml	332.5±364.2	405.0±169.7	146.7±187.2	135.0±141.2
TPCP35PP/ml	5.0±7.1	5.4±7.1	31.7±54.8	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	100.5±140.9	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	10.0±12.7	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	3.2±1.7	3.7±2.1
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	.02±0.02	.09±0.12	.01±0.02	.02±.04
Temperature (°C)	20.3±0.4	21.3±1.1	21.7±1.5	22.5±0.0
Dissolved Oxygen (ppm)	8.2±1.1	7.3±0.3	7.7±0.9	7.6±0.4
pH	7.4±0.0	7.6±0.1	7.5±0.2	7.5±0.2
E.C. (μmhos)	710.0±28.3	620.0±155.0	645.0±63.6	560.0±56.6
Redox (mv)	115.0±7.1	110.0±35.4	150.0±60.8	110.0±0.0
Turbidity (NTU)	10.9±13.6	5.4±3.7	1.6±0.6	2.7±1.6

TABLE A7. PARAMETERS MEASURED FROM JUNE 1978 THROUGH APRIL 1980
AT WELL #6321, GARDEN GROVE

Parameter	1978 June	MONTH July	August	September	October	November	December
TPC35SP/ml	-	-	-	-	-	-	-
TPCP35SP/ml	-	-	-	-	-	-	-
TPC35PP/ml	-	-	-	-	-	-	-
TPCP35PP/ml	-	-	-	-	-	-	-
TC-MF-U/100ml	-	-	-	-	-	-	-
TC-MF-C/ml	-	-	-	-	-	-	-
M-FC/100ml	-	-	-	-	-	-	-
PRES-COL/100ml	-	-	-	-	-	-	-
CONF-COL/100ml	-	-	-	-	-	-	-
Fecal-EC/100ml	-	-	-	-	-	-	-
Residual Chlorine	-	-	-	-	-	-	-
Temperature (^{o}C)	-	-	-	-	-	-	-
Dissolved Oxygen (ppm)	-	-	-	-	-	-	-
pH	-	-	-	-	-	-	-
E.C. (μmhos)	-	-	-	-	-	-	-
Redox (mv)	-	-	-	-	-	-	-
Turbidity (NTU)	-	-	-	-	-	-	-

(continued)

TABLE A7 (continued

Parameter	1979 January	MONTH February	March	April	May
TPC35SP/ml	60.0±0.0	-	-	18.3±2.9	140.0±0.0
TPCP35SP/ml	30.0±0.0	-	-	0.3±0.5	0.0±0.0
TPC35PP/ml	65.0±0.0	-	-	29.7±21.1	135.0±0.0
TPCP35PP/ml	0.0±0.0	-	-	0.0±0.0	0.0±0.0
TC-MF-U/100ml	8.0±0.0	-	-	0.5±0.7	<1.0±0.0
TC-MF-C/ml	<1.0±0.0	-	-	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	-	-	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.0±0.0	-	-	<2.0±0.0	<2.0±0.0
CONF-COL/100ml	<2.0±0.0	-	-	<2.0±0.0	<2.0±0.0
Fecal-EC/100ml	<2.0±0.0	-	-	<2.0±0.0	<2.0±0.0
Residual Chlorine	-	-	-	0.0±0.0	0.0±0.0
Temperature (°C)	17.0±0.0	-	-	19.0±0.0	19.0±0.0
Dissolved Oxygen (ppm)	5.4±0.0	-	-	-	8.3±0.0
pH	7.1±0.0	-	-	7.9±0.5	7.9±0.0
E.C. (μmhos)	760.0±0.0	-	-	791.7±372.4	655.0±0.0
Redox (mv)	160.0±0.0	-	-	60.0±56.6	150.0±0.0
Turbidity (NTU)	0.2±0.0	-	-	0.3±0.1	0.1±0.0

(continued)

TABLE A7 (continued)

	1979	MONTH			
Parameter	June	July	August	September	October
TPC35SP/ml	65.0±0.0	230.0±0.0	80.0±0.0	800.0±0.0	43.0±0.0
TPCP35SP/ml	0.0±0.0	157.0±0.0	20.0±0.0	775.0±0.0	20.0±0.0
TPC35PP/ml	45.0±0.0	190.0±0.0	55.0±0.0	680.0±0.0	25.0±0.0
TPCP35PP/ml	0.0±0.0	50.0±0.0	0.0±0.0	650.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	19.0±0.0	19.0±0.0	19.5±0.0	20.0±0.0	23.0±0.0
Dissolved Oxygen (ppm)	6.0±0.0	6.8±0.0	8.0±0.0	8.5±0.0	7.9±0.0
pH	7.1±0.0	7.3±0.0	7.1±0.0	7.3±0.0	7.6±0.0
E.C. (μmhos)	670.0±0.0	680.0±0.0	-	550.0±0.0	680.0±0.0
Redox (mv)	110.0±0.0	105.0±0.0	180.0±0.0	100.0±0.0	100.0±0.0
Turbidity (NTU)	0.1±0.0	0.2±0.0	0.4±0.0	0.2±0.0	0.2±0.0

(continued)

TABLE A7 (continued)

	1979		1980	MONTH		
Parameter	November	December	January	February	March	April
TPC35SP/ml	50.0±0.0	65.0±0.0	80.0±0.0	70.0±0.0	70.0±0.0	10.0±0.0
TPCP35SP/ml	0.0±0.0	25.0±0.0	0.0±0.0	0.0±0.0	13.0±0.0	5.0±0.0
TPC35PP/ml	25.0±0.0	20.0±0.0	30.0±0.0	20.0±0.0	0.0±0.0	30.0±0.0
TPCP35PP/ml	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF/C/ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF/COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal/EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	19.0±0.0	19.0±0.0	19.0±0.0	18.0±0.0	19.0±0.0	18.0±0.0
Dissolved Oxygen (ppm)	8.8±0.0	8.0±0.0	8.0±0.0	8.2±0.0	7.7±0.0	8.0±0.0
pH	6.9±0.0	6.0±0.0	7.0±0.0	6.5±0.0	7.5±0.0	7.1±0.0
E.C. (μmhos)	575.0±0.0	470.0±0.0	460.0±0.0	485.0±0.0	410.0±0.0	435.0±0.0
Redox (mv)	130.0±0.0	-	100.0±0.0	170.0±0.0	110.0±0.0	130.0±0.0
Turbidity (NTU)	0.3±0.0	0.3±0.0	0.2±0.0	0.5±0.0	0.2±0.0	0.4±0.0

TABLE A8. PARAMETERS MEASURED FROM JUNE 1978 THROUGH APRIL 1980 AT WELL #6064, GARDEN GROVE

Parameter	1978	MONTH					
	June	July	August	September	October	November	December
TPC35SP/ml	-	-	-	-	-	-	-
TPCP35SP/ml	-	-	-	-	-	-	-
TPC35PP/ml	-	-	-	-	-	-	-
TPCP35PP/ml	-	-	-	-	-	-	-
TC-MF-U/100ml	-	-	-	-	-	-	-
TC-MF-C/ml	-	-	-	-	-	-	-
M-FC/100ml	-	-	-	-	-	-	-
PRES-COL/100ml	-	-	-	-	-	-	-
CONF-COL/100ml	-	-	-	-	-	-	-
Fecal-EC/100ml	-	-	-	-	-	-	-
Residual Chlorine	-	-	-	-	-	-	-
Temperature (^{o}C)	-	-	-	-	-	-	-
Dissolved Oxygen (ppm)	-	-	-	-	-	-	-
pH	-	-	-	-	-	-	-
E.C. (μmhos)	-	-	-	-	-	-	-
Redox (mv)	-	-	-	-	-	-	-
Turbidity (NTU)	-	-	-	-	-	-	-

(continued)

TABLE A8 (continued)

Parameter	1979 January	MONTH February	March	April	May
TPC35SP/ml	360.0±0.0	-	-	290.0±407.5	-
TPCP35SP/ml	200.0±0.0	-	-	163.3±282.9	-
TPC35PP/ml	265.0±0.0	-	-	349.3±542.2	150.0±0.0
TPCP35PP/ml	35.0±0.0	-	-	11.7±16.1	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	-	-	0.3±0.0	<1.0±0.0
TC-MF-C/100ml	1.0±0.0	-	-	1.0±0.0	1.0±0.0
M-FC/ 100ml	1.0±0.0	-	-	1.0±0.0	1.0±0.0
PRES-COL/100ml	2.2±0.0	-	-	2.2±0.0	2.2±0.0
CONF-COL/100ml	2.2±0.0	-	-	2.2±0.0	2.2±0.0
Fecal-EC/100ml	2.2±0.0	-	-	2.2±0.0	2.2±0.0
Residual Chlorine (ppm)	0.0±0.0	-	-	0.0±0.0	0.0±0.0
Temperature (^{o}C)	17.0±0.0	-	-	18.3±0.0	18.5±0.0
Dissolved oxygen (ppm)	6.3±0.0	-	-	-	8.2±0.0
pH	7.4±0.0	-	-	7.9±0.4	8.0±0.0
E.C. (μmhos)	800.0±0.0	-	-	903.3±431.5	700.0±0.0
Redox (mv)	150.0±0.0	-	-	175.0±106.1	70.0±0.0
Turbidity (NTU)	0.2±0.0	-	-	0.2±0.1	0.1±0.0

(continued)

TABLE A8. (continued)

Parameter	1979 June	July	MONTH August	1980 September	October	November
TPC35SP/ml	1595.0±0.0	10.0±0.0	10.0±0.0	470.0±0.0	40.0±0.0	40.0±0.0
TPCP35SP/ml	505.0±0.0	0.0±0.0	10.0±0.0	25.0±0.0	20.0±0.0	0.0±0.0
TPC35PP/ml	1255.0±0.0	20.0±0.0	20.0±0.0	270.0±0.0	30.0±0.0	20.0±0.0
TPCP35PP/ml	65.0±0.0	0.0±0.0	0.0±0.0	5.0±0.0	15.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	11.0±0.0	20.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	20.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	8.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	.02±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	18.0±0.0	18.0±0.0	18.0±0.0	19.0±0.0	21.0±0.0	18.0±0.0
Dissolved Oxygen (ppm)	6.9±0.0	8.0±0.0	8.0±0.0	8.4±0.0	6.9±0.0	8.2±0.0
pH	7.1±0.0	7.4±0.0	7.9±0.0	7.3±0.0	7.6±0.0	6.9±0.0
E.C. (μmhos)	730.0±0.0	-	-	580.0±0.0	650.0±0.0	560.0±0.0
Redox (mv)	120.0±0.0	110.0±0.0	180.0±0.0	100.0±0.0	130.0±0.0	120.0±0.0
Turbidity (NTU)	6.4±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0

(continued)

TABLE A8. (continued)

Parameter	1980	MONTH			
	December	January	February	March	April
TPC35SP/ml	55.0±0.0	263.0±0.0	115.0±0.0	160.0±0.0	14.0±0.0
TPCP35SP/ml	0.0±0.0	15.0±0.0	10.0±0.0	97.0±0.0	0.0±0.0
TPC35PP/ml	35.0±0.0	75.0±0.0	20.0±0.0	70.0±0.0	40.0±0.0
TPCP35PP/ml	15.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (°C)	18.5±0.0	17.5±0.0	17.5±0.0	17.0±0.0	19.0±0.0
Dissolved Oxygen (ppm)	7.4±0.0	7.6±0.0	7.4±0.0	7.1±0.0	5.4±0.0
pH	7.3±0.0	6.8±0.0	6.5±0.0	7.6±0.0	7.0±0.0
E.C. (μmhos)	500.0±0.0	480.0±0.0	610.0±0.0	430.0±0.0	380.0±0.0
Redox (mv)	-	130.0±0.0	170.0±0.0	150.0±0.0	155.0±0.0
Turbidity (NTU)	0.4±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.4±0.0

TABLE A9. PARAMETERS MEASURED FROM JUNE 1978 THROUGH SEPTEMBER 1979 AT ARBOR LANE, IRVINE

	1978		MONTH			
Parameter	June	July	August	September	October	November
TPC35SP/ml	1000.0±0.0	180.0±0.0	27.5±31.8	505.0±700.0	53.0±0.0	65.0±7.1
TPCP35SP/ml	-	-	-	-	0.0±0.0	5.0±7.1
TPC35PP/ml	-	-	-	-	-	5.0±0.0
TPCP35PP/ml	-	-	-	-	-	0.0±0.0
TC-MF-U/100ml	-	-	-	55.0±77.8	34.0±0.0	<1.0±0.0
TC-MF-C/100ml	-	-	-	6.0±8.5	-	<1.0±0.0
M-FC/100ml	-	-	-	-	-	<1.0±0.0
PRES-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.45±0.35	0.4±0.0	0.58±0.18
Temperature (^{o}C)	27.0±0.0	25.0±0.0	25.0±0.0	24.0±0.0	23.0±0.0	19.8±1.1
Dissolved Oxygen (ppm)	8.3±0.0	-	8.7±0.4	8.1±1.1	8.4±0.0	9.4±0.2
pH	7.9±0.0	-	8.1±0.0	6.0±0.0	6.1±0.0	7.8±0.4
E.C. (µmhos)	1200.0±0.0	1050.0±0.0	1190.0±14.1	1162.0±0.0	1240.0±0.0	1275.0±35.4
Redox (mv)	-	-	185.0±0.0	-	-40.0±0.0	95.0±417.2
Turbidity (NTU)	1.0±0.0	8.6±0.0	36.0±46.7	0.6±0.6	0.3±0.0	0.8±0.8

continued

TABLE A9. (continued)

	1978	MONTH	1979		
Parameter	December	January	February	March	April
TPC35SP/ml	70.0±0.0	104.0±121.6	24.5±121.6	12.0±11.3	273.3±434.5
TPCP35SP/ml	55.0±0.0	89.5±113.8	0.0±0.0	5.0±7.1	11.7±16.1
TPC35PP/ml	25.0±0.0	127.5±152.0	154.5±205.8	49.0±0.0	245.3±402.4
TPCP35PP/ml	0.0±0.0	57.5±81.3	31.5±40.3	6.0±0.0	2.0±2.6
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<0.3±0.6
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.55±0.0	0.58±0.6	0.81±0.7	0.67±0.1	0.73±0.04
Temperature (^{o}C)	14.5±0.0	13.3±0.4	13.0±0.7	13.5±0.7	16.8±1.3
Dissolved Oxygen (ppm)	6.5±0.0	8.4±2.9	11.9±0.4	12.6±0.0	-
pH	6.5±0.0	8.5±1.1	8.01±0.1	8.1±0.1	8.1±0.1
E.C. (μmhos)	1752.0±0.0	905.0±572.8	1140.0±28.3	1215.0±544.5	1258.3±685.7
Redox (mv)	120.0±0.0	390.0±56.6	300.0±0.0	15.0±275.8	190.0±72.1
Turbidity (NTU)	1.8±0.0	2.6±2.8	0.7±0.1	0.7±0.1	1.6±1.2

(continued)

TABLE A9. (continued)

	1979	MONTH			
Parameter	May	June	July	August	September
TPC35SP/ml	732.5±894.5	195.0±247.5	322.5±307.6	111.7±68.1	115.0±35.4
TPCP35SP/ml	32.5±46.0	70.0±99.0	110.0±148.5	46.7±46.5	67.5±17.7
TPC35PP/ml	480.0±473.8	87.5±95.5	65.0±77.8	73.3±58.0	55.0±58.6
TPCP35PP/ml	10.0±14.1	25.0±35.4	0.0±0.0	35.0±52.2	22.5±17.7
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	3.0±2.8	<1.0±0.0	1.5±0.7
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	2.5±2.1	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.74±0.06	0.80±0.14	0.65±0.09	0.83±0.12	0.86±0.0
Temperature (°C)	19.3±1.8	21.8±0.4	23.7±1.6	25.7±0.3	26.0±0.0
Dissolved Oxygen (ppm)	9.2±0.0	8.7±0.0	85.0±0.07	8.2±0.2	7.7±0.3
pH	8.3±0.1	7.9±0.2	7.8±0.0	8.0±0.4	8.2±0.0
E.C. (μmhos)	920.0±42.4	845.0±7.1	700.0±113.0	920.0±169.8	740.0±14.1
Redox (mv)	160.0±84.9	140.0±42.4	130.0±778.0	206.7±37.9	190.0±0.0
Turbidity (NTU)	0.4±0.1	4.0±4.8	1.7±1.7	4.0±4.5	3.1±2.8

TABLE A10. PARAMETERS MEASURED FROM JUNE 1978 THROUGH APRIL 1980
AT L. PARKVIEW LANE, IRVINE

	MONTH					
Parameter	1978 June	July	August	September	October	November
TPC35SP/ml	10000.0±0.0	1550.0±0.0	115.0±148.5	143.0±89.1	120.1±14.1	15.0±21.2
TPCP35SP/ml	-	-	-	-	11.0±8.5	2.5±3.5
TPC35PP/ml	-	-	-	-	-	26.0±0.0
TPCP25PP/ml	-	-	-	-	-	9.0±0.0
TC-MF-U/100ml	-	-	-	<1.0±0.0	2.0±2.8	<1.0±0.0
TC-MF-C/100ml	-	-	-	-	-	<1.0±0.0
M-FC/100ml	-	-	-	<1.0±0.0	-	<1.0±0.0
PRES-COL/100ml	-	-	5.1±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.13±0.04	0.24±0.05	0.49±0.06
Temperature (^{o}C)	27.0±0.0	24.0±0.0	25.8±0.4	25.0±0.0	23.3±0.4	20.5±2.1
Dissolved Oxygen (ppm)	1.1±0.0	-	8.3±0.0	8.4±1.1	8.0±0.4	9.3±0.4
pH	7.2±0.0	-	8.2±0.1	6.0±0.0	-	8.2±0.6
E.C. (mhos)	1100.0±0.0	1028.0±0.0	1040.0±84.9	994.0±0.0	-	1324.0±135.8
Redox (mv)	-	-	160.0±0.0	-	-22.5±180.3	185.0±247.5
Turbidity (NTU)	50.0±0.0	20.0±0.0	1.4±0.0	0.7±0.4	1.1±0.2	0.9±0.5

(continued)

TABLE A10. (continued)

	1978	1979	MONTH		
Parameter	December	January	February	March	April
TPC35SP/ml	347.5±463.1	22.0±16.9	48.0±16.9	325.0±417.2	310.0±243.3
TPCP35SP/ml	27.5±31.8	8.5±4.9	17.5±24.7	10.0±14.1	80.0±78.1
TPC35PP/ml	25.0±21.2	18.5±19.1	31.0±8.5	363.0±427.1	162.3±123.8
TPCP35PP/ml	5.0±7.1	5.5±0.7	13.5±0.7	17.0±24.0	31.7±20.2
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	0.3±0.6
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	5.7±4.9	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	5.7±4.9	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.6±0.0	0.61±0.04	0.83±0.03	0.71±0.08	0.73±0.03
Temperature (°C)	14.8±1.1	13.0±0.0	12.5±0.6	12.2±1.1	16.3±1.2
Dissolved Oxygen (ppm)	7.7±3.5	8.5±1.1	12.0±0.7	12.7±0.0	-
pH	7.3±1.1	7.7±0.3	8.0±0.3	8.0±0.0	8.0±0.1
E.C. (µmhos)	1252.0±5.7	1178.5±464.6	1065.0±91.9	1225.0±615.2	1100.0±350.0
Redox (mv)	165.0±7.1	390.0±42.4	150.0±0.0	190.0±0.0	186.7±56.9
Turbidity (NTU)	0.9±0.1	0.8±0.6	1.0±0.7	0.6±0.4	1.0±0.6

(continued)

TABLE A10. (continued)

Parameter	1979 May	June	MONTH July	August	September	October
TPC35SP/ml	102.5±116.7	77.5±24.7	187.5 173.2	110.0 95.4	252.2 265.2	132.5 81.4
TPCP35SP/ml	37.5±24.7	65.0±21.2	70.0 42.4	38.3 11.5	35.0 42.4	62.5 45.9
TPC35PP/ml	52.5±53.0	55.0±49.5	57.5 24.7	163.3 93.1	35.0 21.2	51.5 2.1
TC-MF-U/100ml	19.5±14.8	0.0±0.0	0.0 0.0	26.7 7.6	17.5 24.7	12.5 17.7
TC-MF-C/100ml	<1.0±0.0	2.0±2.8	<1.0 0.0	<1.0 0.0	<1.0 0.0	<1.0 0.0
M-FC/100ml	<1.0±0.0	0.5±0.7	<1.0 0.0	<1.0 0.0	<1.0 0.0	<1.0 0.0
PRES-COL/100ml	<1.0±0.0	<1.0±0.0	<1.2 0.0	<2.2 0.0	<2.2 0.0	<2.2 0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2 0.0	<2.2 0.0	<2.2 0.0	<2.2 0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2 0.0	<2.2 0.0	<2.2 0.0	<2.2 0.0
Residual Chlorine	<2.2±0.0	<2.2±0.0	0.64 0.08	0.76 0.15	0.82 0.0	0.66 0.05
Temperature (°C)	0.65±0.01	0.61±0.08	23.5 0.7	25.2 0.3	25.8 0.4	23.0 1.4
Dissolved Oxygen (ppm)	18.9±0.8	21.3±1.1	8.5 0.07	8.2 0.09	8.4 0.6	8.2 0.6
pH	10.4±0.0	9.5±0.3	7.9 0.1	7.9 0.2	8.1 0.03	7.9 0.3
E.C. (µmhos)	8.0±0.5	7.9±0.1	915.0 332.0	770.0 42.4	740.0 0.0	800.0 184.0
Redox (mv)	920.0±42.4	760.0±84.9	87.5 24.7	151.7 93.6	-	170.0 42.4
Turbidity (NTU)	0.4±0.1	3.5±3.8	0.65 0.2	1.1 0.6	1.85 0.9	0.8 0.5

(continued)

TABLE A10. (continued)

	1979		MONTH	1980		
Parameter	November	December	January	February	March	April
TPC35SP/ml	40.0±0.0	20.0±0.0	90.0±0.0	-	75.0±0.0	50.0±0.0
TPCP35PP/ml	0.0±0.0	0.0±0.0	15.0±0.0	-	30.0±0.0	10.0±0.0
TPC35PP/ml	15.0±0.0	20.0±0.0	20.0±0.0	-	40.0±0.0	0.0±0.0
TPCP35PP/ml	5.0±0.0	0.0±0.0	0.0±0.0	-	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	-	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	-	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	-	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	16.0±0.0	-	5.1±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	16.0±0.0	-	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	-	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.64±0.0	0.52±0.0	0.35±0.0	-	0.63±0.0	-
Temperature (°C)	20.0±0.0	16.5±0.0	13.5±0.0	-	15.0±0.0	16.0±0.0
Dissolved Oxygen (ppm)	9.2±0.0	10.1±0.0	10.6±0.0	-	10.2±0.0	11.4±0.0
pH	7.3±0.0	6.4±0.0	7.5±0.0	-	7.5±0.0	7.7±0.0
E.C. (μmhos)	750.0±0.0	780.0±0.0	620.0±0.0	-	395.0±0.0	490.0±0.0
Redox (mv)	240.0±0.0	-	400.0±0.0	-	520.0±0.0	515.0±0.0
Turbidity (NTU)	0.3±0.0	0.6±0.0	2.0±0.0	-	1.5±0.0	0.8±0.0

TABLE A11. PARAMETERS MEASURED FROM JUNE 1978 THROUGH APRIL 1980 AT PASEO PICASSO, IRVINE

	1978		MONTH			
Parameter	June	July	August	September	October	November
TPC35SP/ml	10000.0±0.0	600.0±0.0	5.0±7.1	50.0±28.3	112.5±130.8	102.5±123.7
TPCP35SP/ml	-	-	-	-	88.5±111.0	30.0±35.4
TPC35PP/ml	-	-	-	-	-	14.0±0.0
TPCP35PP/ml	-	-	-	-	-	4.0±0.0
TC-MF-U/100ml	-	-	-	3.0±2.8	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	-	-	-	-	-	<1.0±0.0
M-FC/100ml	-	-	-	-	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.28±0.1	0.55±0.1	0.57±0.2
Temperature (°C)	26.0±0.0	-	25.3±1.1	23.5±0.7	23.0±0.0	20.3±1.8
Dissolved Oxygen (ppm)	6.2±0.0	-	8.7±0.4	8.2±0.1	8.1±1.1	8.9±0.4
pH	7.7±0.0	-	8.1±0.1	7.4±1.9	7.1±0.6	7.3±0.9
E.C. (μmhos)	1500.0±0.0	-	1102.0±31.8	1089.0±0.0	1209.0±43.8	1204.0±14.8
Redox (mv)	-	-	175.0±0.0	-	7.5±137.9	115.0±247.5
Turbidity (NTU)	1.9±0.0	-	2.2±0.0	1.6±0.3	1.6±0.9	1.2±0.6

(continued)

TABLE A11. (continued)

Parameter	1978 December	January	MONTH February	1979 March	April	May
TPC35SP/ml	327.5±399.5	44.8±56.9	45.0±56.5	72.5±99.7	83.7±62.9	37.5±10.6
TPCP35SP/ml	5.0±7.1	1.3±2.5	31.5±43.1	15.0±21.2	27.7±29.4	2.5±3.5
TPC35PP/ml	10.0±0.0	44.7±45.8	12.0±7.1	117.0±117.4	58.7±39.2	36.0±36.8
TPCP35PP/ml	0.0±0.0	4.5±7.1	4.0±2.8	8.0±11.3	26.7±16.5	17.5±24.7
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<0.5±0.7	<1.0±0.0	-
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.56±0.84	0.68±0.07	0.69±0.04	0.61±0.16	0.61±0.02	0.70±0.06
Temperature (°C)	16.0±1.4	13.5±0.4	13.0±0.0	13.8±1.1	17.0±1.0	19.3±1.1
Dissolved Oxygen (ppm)	8.5±2.1	9.5±1.8	11.8±0.8	12.0±0.0	-	9.6±0.0
pH	-	7.5±0.3	7.9±0.2	7.8±0.3	8.1±0.2	8.1±0.2
E.C. (μmhos)	1231.5±86.9	1130.0±243.1	1050.0±0.0	1352.0±746.0	803.3±189.0	905.0±106.1
Redox (mv)	167.5±86.9	390.0±29.4	325.0±0.0	325.0±134.4	190.0±55.7	102.5±10.6
Turbidity (NTU)	1.0±0.9	0.7±0.6	0.9±0.0	0.8±0.8	1.1±0.6	0.4±0.0

(continued)

TABLE A11. (continued)

	1979		MONTH	1980		
Parameter	June	July	August	September	October	November
TPC35SP/ml	35.0±28.3	67.5±81.3	70.0±60.8	15.0±7.0	117.5±166.2	440.0±0.0
TPCP35SP/ml	17.5±24.7	2.5±3.5	15.0±25.9	7.5±10.6	107.5±152.0	30.0±0.0
TPC35PP/ml	15.0±0.0	30.0±42.4	15.0±15.0	5.0±7.1	95.0±134.4	295.0±0.0
TPCP35PP/ml	0.0±0.0	0.0±0.0	0.0±0.0	5.0±7.1	40.0±56.6	0.0±0.0
TC-MF-U/100ml	-	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	16.0±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	16.0±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	16.0±0.0
Residual Chlorine	0.75±0.11	0.56±0.14	0.61±0.55	0.85±0.35	0.68±0.0	0.5±0.0
Temperature (°C)	21.8±1.1	24.0±0.7	25.7±0.3	24.7±0.35	24.0±1.4	21.0±0.0
Dissolved Oxygen (ppm)	9.0±0.3	8.4±0.3	8.4±0.2	8.3±0.14	8.7±0.3	9.2±0.0
pH	7.9±0.1	7.8±0.7	8.1±0.2	8.1±0.2	8.0±0.0	8.0±0.0
E.C. (μmhos)	800.0±63.6	735.0±176.8	855.0±0.0	715.0±49.5	780.0±56.5	700.0±0.0
Redox (mv)	20.0±113.1	130.0±77.8	205.0±40.9	170.0±0.0	155.0±77.8	230.0±0.0
Turbidity (NTU)	1.1±0.7	0.8±0.0	0.9±0.4	1.5±1.8	0.6±0.0	0.4±0.0

(continued)

TABLE A11. (continued)

Parameter	1979 December	January	MONTH 1980 February	March	April
TPC35SP/ml	460.0±0.0	620.0±0.0	40.0±0.0	55.0±0.0	320.0±0.0
TPCP35SP/ml	0.0±0.0	55.0±0.0	5.0±0.0	0.0±0.0	0.0±0.0
TPC35PP/ml	55.0±0.0	105.0±0.0	10.0±0.0	30.0±0.0	220.0±0.0
TPCP35PP/ml	5.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	16.0±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	9.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.32±0.0	0.5±0.0	0.47±0.0	0.9±0.0	-
Temperature (°C)	17.5±0.0	14.5±0.0	14.5±0.0	15.5±0.0	16.0±0.0
Dissolved Oxygen (ppm)	10.0±0.0	11.0±0.0	10.8±0.0	10.8±0.0	123.0±0.0
pH	6.2±0.0	7.6±0.0	7.5±0.0	7.6±0.0	7.2±0.0
E.C. (μmhos)	760.0±0.0	610.0±0.0	665.0±0.0	495.0±0.0	520.0±0.0
Redox (mv)	280.0±0.0	375.0±0.0	420.0±0.0	550.0±0.0	450.0±0.0
Turbidity (NTU)	1.5±0.0	1.6±0.0	2.6±0.0	0.9±0.0	1.0±0.0

TABLE A12. PARAMETERS MEASURED FROM JUNE 1978 THROUGH SEPTEMBER 1979 AT VIA MESSINA, IRVINE

	MONTH				
Parameter	1978 June	July	August	September	October
TPC35SP/ml	-	232.0±0.0	15.0±21.2	-	174.0±97.6
TPCP35SP/ml	-	-	-	-	40.0±14.1
TPC35PP/ml	-	-	-	-	-
TPCP35PP/ml	-	-	-	-	-
TC-MF-U/100ml	-	-	-	1.5±2.1	3.5±4.9
TC-MF-C/ml	-	-	-	<1.0±0.0	-
M-FC/100ml	-	-	-	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	-	<2.0±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	-	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	-	-	0.10±0.0	1.33±0.18
Temperature (°C)	24.5±0.0	24.0±0.0	25.8±0.0	24.3±0.4	23.5±0.7
Dissolved Oxygen (ppm)	4.6±0.0	-	8.4±0.3	7.4±0.0	8.4±0.5
pH	7.2±0.0	-	8.1±0.1	6.5±0.0	7.4±0.0
E.C. (µmhos)	1300.0±0.0	990.0±0.0	1040.0±84.9	1099.0±0.0	1267.0±38.9
Redox (mv)	200.0±0.0	-	127.0±0.0	-10.0±49.5	32.5±109.6
Turbidity (NTU)	1.9±0.0	14.0±0.0	1.4±0.0	1.1±0.4	1.1±0.9

(continued)

TABLE A12. (continued)

	1978	MONTH	1979		
Parameter	November	December	January	February	March
TPC35SP/ml	145.0±0.0	152.5±38.9	300.5±410.8	1367.0±1743.0	113.5±23.3
TPCP35SP/ml	5.0±0.0	27.5±24.7	7.5±3.5	43.5±79.9	29.0±41.0
TPC35PP/ml	320.0±0.0	525.0±544.4	126.0±178.2	321.5±259.5	88.5±64.3
TPCP35PP/ml	50.0±0.0	0.0±0.0	4.0±5.7	60.5±70.0	45.5±64.3
TC-MF-U/100ml	12.0±0.0	12.0±17.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	12.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	14.3±0.0	<<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	.05±0.0	0.1±0.1	.26±0.37	.36±0.44	.56±0.08
Temperature (^{o}C)	20.0±0.0	17.5±2.1	14.5±0.7	14.0±0.7	14.5±0.07
Dissolved Oxygen (ppm)	9.3±0.0	8.1±2.3	8.3±2.1	10.9±0.7	11.8±0.0
pH	8.3±0.0	7.0±0.0	8.1±0.7	8.0±0.1	8.0±0.4
E.C. (μmhos)	1196.0±0.0	1410.5±152.0	1100.0±438.4	1035.0±0.0	1260.0±551.5
Redox (mv)	50.0±0.0	77.5±24.7	280.0±127.3	35.0±0.0	275.0±91.9
Turbidity (NTU)	1.3±0.0	2.0±0.0	0.7±0.3	0.8±0.0	0.6±0.2

(continued)

TABLE A12. (continued)

1979	MONTH					
Parameter	April	May	June	July	August	September
TPC35SP/ml	58.3±56.2	172.5±53.0	22.5±31.8	32.5±38.9	98.3±140.2	107.5±109.6
TPCP35SP/ml	5.0±5.0	37.5±31.8	12.5±17.7	0.0±0.0	10.0±17.4	37.5±53.0
TPC35PP/ml	94.0±74.6	585.0±707.1	15.0±0.0	25.0±14.1	58.3±53.9	20.0±7.1
TPCP35PP/ml	10.0±7.0	200.0±254.0	0.0±0.0	0.0±0.0	5.0±8.7	5.0±0.0
TC-MF-U/100ml	<1.0±0.0	4.0±5.7	<1.0±0.0	<1.0±0.0	3.7±3.8	5.0±5.7
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	2.6±2.9	1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	7.8±7.3	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	7.8±7.3	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	.44±0.38	.71±0.1	.73±0.13	.53±0.07	.47±0.40	.85±0.0
Temperature (^{o}C)	17.2±0.8	18.9±1.3	21.8±1.1	23.8±0.4	25.3±0.6	25.8±0.4
Dissolved Oxygen (ppm)	9.5±0.0	9.4±0.0	8.6±0.3	8.1±0.1	8.2±0.2	8.2±0.2
pH	8.1±0.2	8.1±0.2	8.2±0.4	7.9±0.1	7.9±0.4	8.1±0.2
E.C. (μmhos)	860.0±52.0	1450.0±664.7	805.0±70.0	870.0±325.0	930.0±381.0	745.0±7.1
Redox (mv)	176.7±63.5	135.0±49.5	80.0±35.4	117.0±45.9	183.0±20.8	420.0±0.0
Turbidity (NTU)	1.1±0.5	.7±0.5	1.4±0.6	3.5±3.5	1.7±0.9	2.5±1.4

TABLE A13. PARAMETERS MEASURED FROM JUNE 1978 THROUGH APRIL 1980 AT IRVINE SOURCE, IRVINE

	1978	MONTH					
Parameter	June	July	August	September	October	November	December
TPC35SP/ml	-	-	-	-	-	-	-
TPCP35SP/ml	-	-	-	-	-	-	-
TPC35PP/ml	-	-	-	-	-	-	-
TPCP35PP/ml	-	-	-	-	-	-	-
TC-MF-U/100ml	-	-	-	-	-	-	-
TC-MF-C/ml	-	-	-	-	-	-	-
M-FC/100ml	-	-	-	-	-	-	-
PRES-COL/100ml	-	-	-	-	-	-	-
CONF-COL/100ml	-	-	-	-	-	-	-
Fecal-EC/100ml	-	-	-	-	-	-	-
Residual Chlorine	-	-	-	-	-	-	-
Temperature (°C)	-	-	-	-	-	-	-
Dissolved Oxygen (ppm)	-	-	-	-	-	-	-
pH	-	-	-	-	-	-	-
E.C. (μmhos)	-	-	-	-	-	-	-
Redox (mv)	-	-	-	-	-	-	-
Turbidity (NTU)	-	-	-	-	-	-	-

(continued)

Table A13 (continued)

Parameter	1979 January	February	March	April	May	June
TPC35SP/ml	315.0±387.9	-	25.0±0.0	22.5±3.5	25.0±0.0	25.0±0.0
TPCP35SP/ml	1.7±2.9	-	20.0±0.0	0.0±0.0	0.0±0.0	5.0±0.0
TPC35PP/ml	65.0±65.5	-	5.0±0.0	5.0±0.0	10.0±0.0	10.0±0.0
TPCP35PP/ml	0.0±0.0	-	1.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	0.6±1.2	-	<1.0±0.0	1.0±1.4	<1.0±0.0	<1.0±0.0
TC-MF-C/ml	<1.0±0.0	-	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	-	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	3.9±2.9	-	2.2±0.0	2.2±0.0	2.2±0.0	2.2±0.0
CONF-COL/100ml	3.2±1.7	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.72±0.5	-	0.83±0.0	0.76±0.04	0.83±0.0	0.65±0.0
Temperature (^{o}C)	12.2±0.3	-	12.5±0.0	16.5±0.7	18.7±0.0	22.0±0.0
Dissolved Oxygen (ppm)	9.3±2.3	-	-	-	10.5±0.7	8.9±0.0
pH	7.8±0.2	-	8.0±0.0	8.1±0.1	8.0±0.0	7.9±0.0
E.C. (μmhos)	1066.6±300.9	-	1080.0±0.0	855.0±49.5	880.0±0.0	865.0±0.0
Redox (mv)	406.6±25.2	-	210.0±0.0	175.0±106.0	90.0±0.0	-
Turbidity (NTU)	0.5±0.06	-	0.2±0.0	0.5±0.1	0.2±0.0	0.2±0.0

(continued)

Table A13 (continued)

	1979					
Parameter	July	August	September	October	November	December
TPC35SP/ml	3.0±0.0	40.0±0.0	5.0±0.0	30.0±0.0	30.0±0.0	10.0±0.0
TPCP35SP/ml	0.0±0.0	0.0±0.0	0.0±0.0	5.0±0.0	15.0±0.0	0.0±0.0
TPC35PP/ml	-	30.0±0.0	25.0±0.0	210.0±0.0	20.0±0.0	20.0±0.0
TPCP35PP/ml	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	16.0±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	.65±0.21	0.9±0.0	1.0±0.0	0.87±0.0	0.71±0.0	0.55±0.0
Temperature (^{o}C)	23.8±1.1	5.0±0.0	25.0±0.0	23.5±0.0	20.0±0.0	15.5±0.0
Dissolved Oxygen (ppm)	8.8±0.4	8.6±0.0	9.2±0.0	9.5±0.0	10.4±0.0	10.3±0.0
pH	8.2±0.2	7.9±0.0	8.1±0.0	8.0±0.0	7.5±0.0	6.3±0.0
E.C. (μmhos)	1047.5±215.7	-	750.0±0.0	860.0±0.0	750.0±0.0	720.0±0.0
Redox (mv)	180.0±14.1	210.0±0.0	190.0±0.0	160.0±0.0	220.0±0.0	-
Turbidity (NTU)	0.4±0.0	1.0±0.0	0.4±0.0	0.3±0.0	0.4±0.0	0.2±0.0

(continued)

Table A13 (continued)

	1980			
Parameter	January	February	March	April
TPC35SP/ml	14.0±0.0	40.0±0.0	23.0±0.0	40.0±0.0
TPCP35SP/ml	7.0±0.0	30.0±0.0	10.0±0.0	0.0±0.0
TPC35PP/ml	0.0±0.0	40.0±0.0	10.0±0.0	30.0±0.0
TPCP35PP/ml	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100 ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.60±0.0	0.6±0.0	0.5±0.0	-
Temperature (^{o}C)	3.0±0.0	3.0±0.0	13.0±0.0	14.5±0.0
Dissolved Oxygen (ppm)	10.9±0.0	11.0±0.0	10.6±0.0	12.2±0.0
pH	7.7±0.0	7.4±0.0	7.7±0.0	7.3±0.0
E.C. (μmhos)	575.0±0.0	720.0±0.0	300.0±0.0	540.0±0.0
Redox (mv)	450.0±0.0	490.0±0.0	475.0±0.0	470.0±0.0
Turbidity (NTU)	0.60±0.0	0.4±0.0	0.3±0.0	0.4±0.0

TABLE A14 . PARAMETERS MEASURED FROM JULY 1979 THROUGH APRIL 1980
AT WELL 23/9, GARDEN GROVE

Parameter	1979 July*	MONTH August	September	October	November
TPC35SP/ml	-	50.0±0.0	10.0±0.0	20.0±0.0	40.0±0.0
TPCP35SP/ml	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TPC35PP/ml	-	10.0±0.0	15.0±0.0	15.0±0.0	20.0±0.0
TPCP35PP/ml	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	-	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	-	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	-	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
CONF-COL/100ml	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Fecal-EC/100ml	-	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (^{o}C)	-	22.5±0.0	24.0±0.0	21.0±0.0	20.0±0.0
Dissolved Oxygen (ppm)	-	8.6±0.0	6.8±0.0	7.4±0.0	8.2±0.0
pH	-	7.1±0.0	7.1±0.0	7.5±0.0	7.5±0.0
E.C. (μmhos)	-	-	750.0±0.0	660.0±0.0	680.0±0.0
Redox (mv)	-	185.0±0.0	240.0±0.0	140.0±0.0	145.0±0.0
Turbidity (NTU)	-	0.4±0.0	0.2±0.0	0.2±0.0	0.2±0.0

(continued)

TABLE A14 (continued)

Parameter	1979 December	January	MONTH February	1980 March	April
TPC35SP/ml	220.0±0.0	35.0±0.0	65.0±0.0	147.0±0.0	10.0±0.0
TPCP35SP/ml	20.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.0±0.0
TPC35PP/ml	10.0±0.0	10.0±0.0	0.0±0.0	107.0±0.0	0.0±0.0
TPCP35PP/ml	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
TC-MF-U/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
TC-MF-C/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
M-FC/100ml	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0	<1.0±0.0
PRES-COL/100ml	9.2±0.0	<2.2±0.0	<2.2±0.0	9.3±0.0	<2.2±0.0
CONF-COL/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	2.2±0.0	<2.2±0.0
Fecal-EC/100ml	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0	<2.2±0.0
Residual Chlorine	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Temperature (^{o}C)	18.0±0.0	17.0±0.0	17.0±0.0	19.5±0.0	19.5±0.0
Dissolved Oxygen (ppm)	7.9±0.0	8.7±0.0	8.0±0.0	9.0±0.0	6.7±0.0
pH	6.0±0.0	6.8±0.0	6.5±0.0	7.5±0.0	6.8±0.0
E.C. (μmhos)	700.0±0.0	560.0±0.0	650.0±0.0	470.0±0.0	480.0±0.0
Redox (mv)	-	140.0±0.0	170.0±0.0	170.0±0.0	130.0±0.0
Turbidity (NTU)	0.3±0.0	0.5±0.0	0.5±0.0	0.2±0.0	0.2±0.0

TABLE A15. MONTHLY MEAN BACTERIAL COUNTS ON R-2A and M-SPC MEDIA AT GARDEN GROVE WATER DISTRIBUTION SITE AND SOURCE LOCATIONS

	1979					
Parameter	January	February	March	April	May	June
			Sandra #2			
Low Nutrient R-2A	675.5±491.4	395.5±154.9	510.0±318.2	433.3±495.6	1080.0±594.0	325.0±275.8
Low Nutrient R-2A Pigmented	97.5±88.4	5.0±7.1	45.0±42.4	16.7±5.8	50.0±49.5	45.0±7.1
M-Standard Plate Count Medium Total	-*	-	901.5±917.1	1543.3±1086.1	2400.0±424.3	935.0±799.0
M-Standard Plate Count Medium Pigmented	-	-	29.0±1.4	256.7±220.5	850.0±212.1	330.0±381.8
			Rockinghorse			
Low Nutrient R-2A	1685.0±403.1	464.5±531.0	940.0±792.0	556.7±432.6	507.5±74.2	225.0±42.4
Low Nutrient R-2A Pigmented	50.0±42.4	65.0±91.9	35.0±49.5	61.7±48.0	45.0±7.1	37.5±31.8
M-Standard Plate Count Medium Total	-	-	475.5±388.3	2730.0±3803.1	850.0±353.6	1300.0±989.9
M-Standard Plate Count Medium Pigmented	-	-	27.0±24.0	120.0±85.4	500.0±141.4	200.0±282.8

(continued)

TABLE **A15.** (continued)

			1979			
Parameter	January	February	March	April	May	June
			Harris			
Low Nutrient R-2A	1190.0±0.0	857.7±17.7	717.5±173.2	503.3±40.1	695.0±622.3	582.5±152.0
Low Nutrient R-2A Pigmented	540.0±0.0	307.5±46.0	275.0±325.3	295.0±75.5	325.0±247.5	115.0±134.4
M-Standard Plate Count Medium Total	-	-	587.5±484.4	1133.3±450.9	2250.0±353.6	1950.0±495.0
M-Standard Plate Count Medium Pigmented	-	-	24.5±26.2	630.0±403.4	1400.0±282.8	100.0±141.4
			Marshall			
Low Nutrient R-2A	975.0±381.8	1375.0±388.9	164.5±36.1	276.7±195.3	632.5±180.3	597.5±569.2
Low Nutrient R-2A Pigmented	75.0±14.1	50.0±70.7	21.5±26.2	13.3±12.6	50.0±56.6	0.0±0.0
M-Standard Plate Count Medium Total	-	-	800.0±396.0	396.7±97.1	950.0±353.6	5230.0±7170.1
M-Standard Plate Count Medium Pigmented	-	-	80.0±42.4	136.7±160.7	450.0±495.0	1315.0±1817.3

(continued)

TABLE A15. (continued)

Parameter	January	February	1979 March	April	May	June
			Well 6064			
Low Nutrient R-2A	600.0±0.0	-	-	401.0±412.9	2720.0±0.0	1045.0±0.0
Low Nutrient R-2A Pigmented	195.0±0.0	-	-	155.0±205.4	2530.0±0.0	0.0±0.0
M-Standard Plate Count Medium Total	-	-	-	1413.3±1833.5	300.0±0.0	1000.0±0.0
M-Standard Plate Count Medium Pigmented	-	-	-	753.3±926.1	300.0±0.0	-
			Well 6321			
Low Nutrient R-2A	130.0±0.0	-	-	20.7±14.0	125.0±0.0	15.0±0.0
Low Nutrient R-2A Pigmented	10.0±0.0	-	-	2.0±2.6	25.0±0.0	0.0±0.0
M-Standard Plate Count Medium Total	-	-	-	26.3±16.5	1200.0±0.0	200.0±0.0
M-Standard Plate Count Medium Pigmented	-	-	-	6.7±5.9	700.0±0.0	180.0±0.0

(continued)

TABLE A15. (continued)

Parameter	July	August	1979 September	October	November	December
			Sandra #2			
Low Nutrient R-2A Total	9151.5±6154.0	768.3±412.6	1157.5±477.3	–	–	–
Low Nutrient R-2A Pigmented	990.0±1145.5	108.3±80.4	535.0±608.1	–	–	–
M-Standard Plate Count Medium Total	6650.0±4737.6	563.3±212.2	395.0±332.3	–	–	–
M-Standard Plate Count Medium Pigmented	2400.0±0.0	3400.0±197.0	75.0±7.1	–	–	–
			Rockinghorse			
Low Nutrient R-2A Total	442.5±505.6	615.0±554.7	4185.0±4688.1	–	520.0±0.0	1770.0±0.0
Low Nutrient R-2A Pigmented	12.5±17.7	143.3±218.1	1347.5±1771.3	–	30.0±0.0	130.0±0.0
M-Standard Plate Count Medium Total	1195.0±1279.9	526.7±521.7	1465.0±1605.1	400.0±0.0	610.0±0.0	900.0±0.0
M-Standard Plate Count Medium Pigmented	450.0±495.0	183.3±190.9	880.0±1018.2	400.0±0.0	250.0±0.0	200.0±0.0

(continued)

TABLE A15. (continued)

Parameter	1980 January	February	March	April
		Harris		
Low Nutrient R-2A	4000.0±0.0	2400.0±0.0	353.0±0.0	1370.0±0.0
Low Nutrient R-2A	3450.0±0.0	2300.0±0.0	213.0±0.0	1300.0±0.0
m-Standard Plate Count Medium Total	8300.0±0.0	9200.0±0.0	8000.0±0.0	18000.0±0.0
m-Standard Plate Count Medium Pigmented	0.0±0.0	9100.0±0.0	7800.0±0.0	12000.0±0.0
		Marshall		
Low Nutrient R-2A	-	-	-	-
Low Nutrient R-2A Pigmented	-	-	-	-
m-Standard Plate Count Medium Total	-	-	-	-
m-Standard Plate Count Medium Pigmented	-	-	-	-

(continued)

TABLE A15. (continued)

	1980			
Parameter	January	February	March	April
		Sandra #2		
Low Nutrient R-2A	-	-	-	-
Low Nutrient R-2A Pigmented	-	-	-	-
M-Standard Plate Count Medium Total	-	-	-	-
M-Standard Plate Count Medium Pigmented	-	-	-	-
		Rockinghorse		
Low Nutrient R-2A	365.0±0.0	490.0±0.0	573.0±0.0	1250.0±0.0
Low Nutrient R-2A Pigmented	35.0±0.0	40.0±0.0	57.0±0.0	73.0±0.0
M-Standard Plate Count Medium Total	350.0±0.0	200.0±0.0	963.0±0.0	590.0±0.0
M-Standard Plate Count Medium Pigmented	290.0±0.0	0.0±0.0	117.0±0.0	120.0±0.0

*- Not determined

TABLE A16. MONTHLY MEAN BACTERIAL COUNTS ON R-2A AND M-SPC MEDIA AT IRVINE WATER DISTRIBUTION SITE AND SOURCE LOCATIONS

	1979					
Parameter	January	February	March	April	May	June
			Paseo Picasso			
Low Nutrient R-2A Total	387.5±445.9	30.5±29.0	145.0±148.5	240.0±49.2	225.0±120.2	117.5±38.9
Low Nutrient R-2A Pigmented	218.7±359.0	5.5±4.9	0.0±0.0	53.3±64.5	57.5±67.2	15.0±21.2
M-Standard Plate Count Medium Total	-	-	180.5±183.1	4900.0±7156.6	370.0±183.8	65.0±21.2
M-Standard Plate Count Medium Pigmented	-	-	53.5±37.5	4250.0±6142.7	320.0±254.6	50.0±14.1
			Via Messina			
Low Nutrient R-2A Total	450.0±629.3	229.0±130.1	150.0±52.3	155.3±66.3	427.5±321.7	90.0±56.6
Low Nutrient R-2A Pigmented	15.0±21.2	17.5±23.3	60.0±80.6	41.7±59.7	147.5±187.4	47.5±67.1
M-Standard Plate Count Medium Total	-	-	110.0±0.0	143.3±100.2	850.0±495.0	185.0±148.5
M-Standard Plate Count Medium Pigmented	-	-	70.0±0.0	86.7±60.3	800.0±565.7	145.0±120.0

(continued)

TABLE A16. (continued)

Parameter	January	February	1979 March	April	May	June
			L. Parkview Lane			
Low Nutrient R-2A Total	38.5±9.2	45.0±19.8	635.5±805.4	448.3±121.7	77.5±74.2	112.5±46.0
Low Nutrient R-2A Pigmented	24.5±7.8	13.0±17.0	40.5±14.8	187.7±156.7	17.5±17.7	30.0±42.4
M-Standard Plate Count Medium Total	-	-	634.0±828.7	734.3±1004.5	160.0±0.0	280.0±0.0
M-Standard Plate Count Medium Pigmented	-	-	6.5±9.2	90.0±130.8	155.0±7.1	270.0±0.0
			Arbor Lane			
Low Nutrient R-2A Total	609.0±758.0	74.0±59.4	76.5±36.1	360.0±33.7	1335.0±834.4	272.5±371.2
Low Nutrient R-2A Pigmented	475.5±649.8	32.5±41.7	7.5±10.6	25.0±21.8	22.5±31.8	0.0±0.0
M-Standard Plate Count Medium Total	-	-	109.5±72.8	2666.7±4102.3	1200.0±707.1	535.0±233.3
M-Standard Plate Count Medium Pigmented	-	-	32.5±2.1	260.0±177.8	300.0±424.3	435.0±233.3

(continued)

TABLE A16. (continued)

Parameter	January	February	1979 March	April	May	June
			Irvine Source			
Low Nutrient R-2A Total	207.7±329.5	-	3.0±0.0	20.0±0.0	60.0±0.0	30.0±0.0
Low Nutrient R-2A Pigmented	66.0±110.0	-	1.0±0.0	5.0±0.0	5.0±0.0	10.0±0.0
M-Standard Plate Count Medium Total	-	-	10.0±0.0	1300.0±1272.8	30.0±0.0	30.0±0.0
M-Standard Plate Count Medium Pigmented	-	-	3.0±0.0	1150.0±1343.5	20.0±0.0	10.0±0.0

(continued)

TABLE A16. (continued)

Parameter	July	August	1979 September	October	November	December
			Paseo Picasso			
Low Nutrient R-2A Total	495.0±431.3	36.7±33.0	72.5±88.4	170.0±0.0	390.0±0.0	665.0±0.0
Low Nutrient R-2A Pigmented	67.5±81.3	0.0±0.0	15.0±21.2	160.0±0.0	10.0±0.0	20.0±0.0
M-Standard Plate Count Medium Total	185.0±148.5	53.3±61.1	55.0±7.1	120.0±0.0	470.0±0.0	2300.0±0.0
M-Standard Plate Count Medium Pigmented	130.0±99.0	13.3±23.1	25.0±35.4	110.0±0.0	60.0±0.0	400.0±0.0
			Via Messina			
Low Nutrient R-2A Total	257.5±166.2	55.0±77.8	547.5±555.1	-	-	-
Low Nutrient R-2A Pigmented	70.0±14.2	27.5±38.9	252.5±314.7	-	-	-
M-Standard Plate Count Medium Total	510.0±240.4	923.3±1453.5	1710.0±1824.3	-	-	-
M-Standard Plate Count Medium Pigmented	455.0±275.8	880.0±1404.7	1535.0±1930.4	-	-	-

(continued)

TABLE A16. (continued)

Parameter	July	August	1979 September	October	November	December
			L. Parkview Lane			
Low Nutrient R-2A Total	207.5±102.5	121.7±119.0	222.5±194.4	195.0±0.0	40.0±0.0	40.0±0.0
Low Nutrient R-2A Pigmented	87.5±53.0	20.0±26.4	47.5±38.9	40.0±0.0	15.0±0.0	15.0±0.0
M-Standard Plate Count Medium Total	380.0±311.1	466.7±302.4	35.0±21.2	180.0±0.0	150.0±0.0	310.0±0.0
M-Standard Plate Count Medium Pigmented	215.0±120.2	393.3±279.4	30.0±14.1	170.0±0.0	70.0±0.0	260.0±0.0
			Arbor Lane			
Low Nutrient R-2A Total	735.0±947.5	605.3±949.8	997.5±1332.9	-	-	-
Low Nutrient R-2A Pigmented	250.0±353.6	81.7±82.5	165.0±205.1	-	-	-
M-Standard Plate Count Medium Total	1000.0±1131.4	686.7±578.4	60.0±28.3	-	-	-
M-Standard Plate Count Medium Pigmented	800.0±1131.4	510.0±441.9	55.0±21.2	-	-	-

(continued)

TABLE A16. (continued)

			1980			
Parameter	January	February	March	April	May	June
			Paseo Picasso			
Low Nutrient R-2A Total	5250.0±0.0	210.0±0.0	100.0±0.0	290.0±0.0	-	-
Low Nutrient R-2A Pigmented	10.0±0.0	60.0±0.0	0.0±0.0	0.0±0.0	-	-
M-Standard Plate Count Medium Total	5400.0±0.0	410.0±0.0	75.0±0.0	410.0±0.0	-	-
M-Standard Plate Count Medium Pigmented	0.0±0.0	410.0±0.0	20.0±0.0	30.0±0.0	-	-
			Via Messina			
Low Nutrient R-2A Total	-	-	-	-	-	-
Low Nutrient R-2A Pigmented	-	-	-	-	-	-
M-Standard Plate Count Medium Total	-	-	-	-	-	-
M-Standard Plate Count Medium Pigmented	-	-	-	-	-	-

(continued)

TABLE A16. (continued)

Parameter	January	February	1980 March	April	May	June
			L. Parkview Lane			
Low Nutrient R-2A Total	240.0±0.0	-	535.0±0.0	130.0±0.0	-	-
Low Nutrient R-2A Pigmented	10.0±0.0	-	55.0±0.0	0.0±0.0	-	-
M-Standard Plate Count Medium Total	210.0±0.0	-	230.0±0.0	140.0±0.0	-	-
M-Standard Plate Count Medium Pigmented	80.0±0.0	-	170.0±0.0	130.0±0.0	-	-
			Arbor Lane			
Low Nutrient R-2A Total	-	-	-	-	-	-
Low Nutrient R-2A Pigmented	-	-	-	-	-	-
M-Standard Plate Count Medium Total	-	-	-	-	-	-
M-Standard Plate Count Medium Pigmented	-	-	-	-	-	-

*- Not determined

APPENDIX B

CHEMICAL INFORMATION AND CHLORINE DEMAND ON TOTAL PLATE COUNT MEDIUM

JAMES M. MONTGOMERY, CONSULTING ENGINEERS, INC.

MEMORANDUM

TO: Larry Y. C. Leong

DATE: August 14, 1979

FILE NO:

FROM: John Cromer

SUBJECT:

CLIENT:

The following is a brief report of the chlorination studies performed on the media sample from Irvine.

EXPERIMENTAL

Two experiments were done.

The first consisted of dosing a series of fifteen - ml aliquots of the media with increasing amounts of a concentrated sodium hypochlorite solution. After a one-hour holding time, the total residual chlorine was determined using the acidic iodine-thiosulfate titration procedure with starch endpoint.

The second experiment involved the use of the amperometric titration procedure for the determination of both free and combined residual chlorine. The media was diluted 1/100 and 200 ml aliquots were dosed with hypochlorite solution. After a thirty minute holding time the chlorinated solutions were titrated with phenylarsine oxide (PAO) to an amperometric dead-stop endpoint at pH 7.0 for free chlorine, at pH 7.0 with iodide added for monochloramine, and at pH 4.0 with iodide added for dichloramine. Total chlorine residual was calculated by summing the endpoints.

RESULTS

Chemical characteristics of the media are given in Table 1. The results of the thiosulfate titrations are given in Table 2 and are plotted in Figure 1. The results of the PAO titrations are given in Table 3 and are plotted in Figure 2.

TABLE 1 - CHEMICAL CHARACTERISTICS OF THE MEDIA

Ammonia nitrogen concentration : 6.1 mg N/l

Organic nitrogen concentration : 213 mg N/l

Conductivity : 392

pH : 6.90

TABLE 2 - TOTAL RESIDUAL CHLORINE MEASURED AS A FUNCTION OF APPLIED CHLORINE. T = 25° C. pH = 6.9. 1.0 HOUR HOLDING TIME. ALL CONCENTRATIONS ARE IN mg Cl/l.

Chlorine Applied	Chlorine Measured
193	80
374	153
545	215
705	295
856	317
998	339
1260	360
1500	394
1710	420
2080	618
2400	882
2660	1099

TABLE 3 - FREE AND COMBINED RESIDUAL CHLORINE MEASURED AS A FUNCTION OF APPLIED CHLORINE. T = 25° C. pH = 6.9. 0.5 HOURS HOLDING TIME. ALL CONCENTRATIONS ARE IN mg Cl/l. 1/100 MEDIA DILUTION

	Chlorine Measured	
Chlorine Applied	Free	Total
10	0.6	6.4
15	3.8	8.7
20	7.0	13.1
25	10.2	17.2
30	15.0	22.0

DISCUSSION

The high organic nitrogen concentration is confirmed in the shape of the breakpoint curve of Figure 1. Since the reactions of chlorine with ammonia are usually completed within thirty minutes (at pH = 6.5 - 8.5) the persistence of a combined residual for longer times indicates the presence of chlorinated organic nitrogen compounds (even though they were titrated as dichloramine). The presence of these more stable compounds precludes an accurate breakpoint determination. While there appears to be an inflection at an applied chlorine concentration of 1900 mg/l, a theoretical breakpoint of 1347 mg/l can be calculated using the experimentally determined organic nitrogen value, assuming the organic nitrogen behaves, mole for mole, similarly to ammonia.

Memo to
Larry Y. C. Leong

August 14, 1979

The amperometric titration results show the development of a free chlorine residual of 60 mg/l at an applied concentration of 1000 mg/l. At higher applied concentrations the free residual continues to increase and becomes more or less linear at applied concentrations greater than 2000 mg/l which is coincidental with the one-to-one linear increase of total chlorine in Figure 1. At points above 2000 mg/l the combined residual remains relatively constant at about 6000 mg Cl/l as the free chlorine residual increases in proportion to the applied chlorine concentration. This point at 2000 mg/l applied chlorine could be termed the breakpoint but since the organic nitrogen is never completely oxidized to nitrogen gas, the use of the term breakpoint is somewhat misleading.

In summary, the high organic nitrogen content reacts with chlorine to form a relatively stable combined chlorine residual which persists even after the establishment of a linearly increasing free chlorine residual. This linear increase begins at about 2000 mg/l applied chlorine, below which the organic-nitrogen-chlorine residual is predominant. With a thirty minute contact time, this combined chlorine residual remains relatively constant at about 6000 mg Cl/l at applied chlorine concentrations greater than 2000 mg/l.

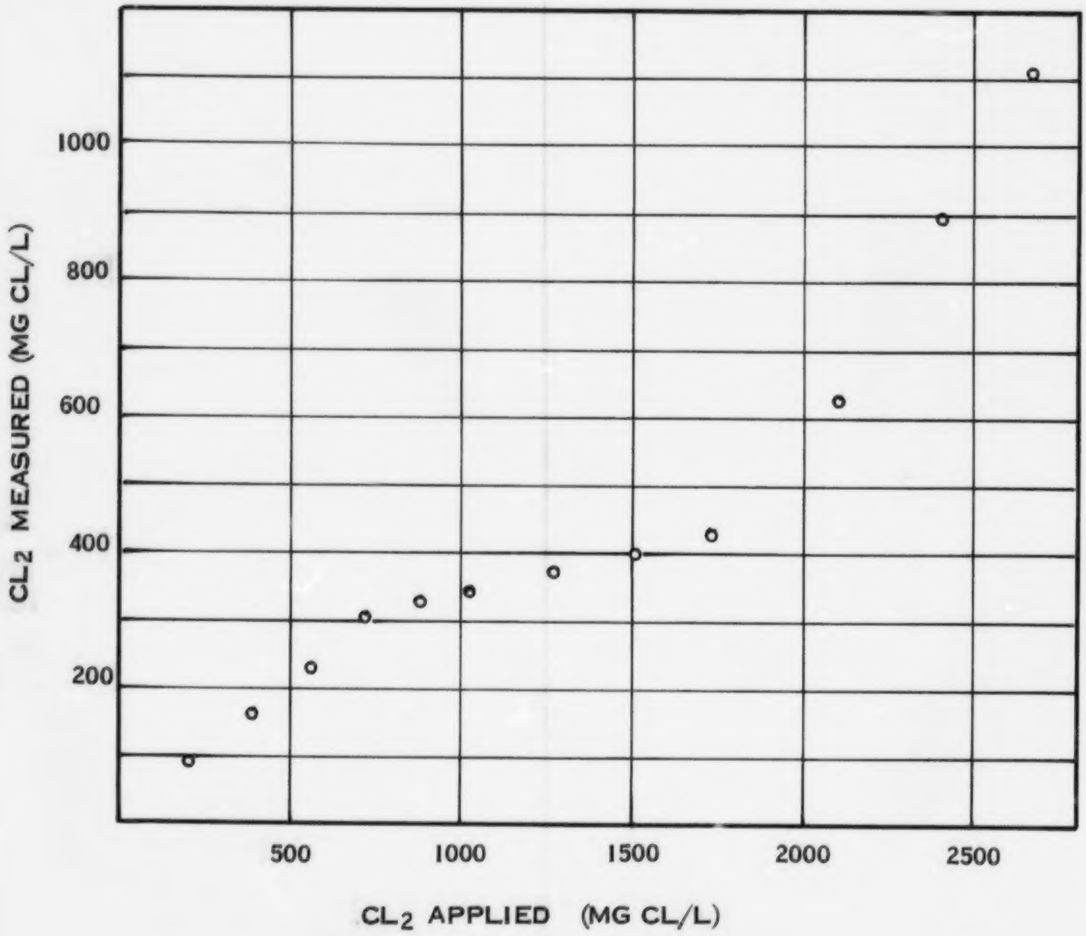


FIGURE B1.

Total Residual Chlorine Concentration measured as a function of applied chlorine after a one hour holding time. T = 25°C. pH = 6.9.

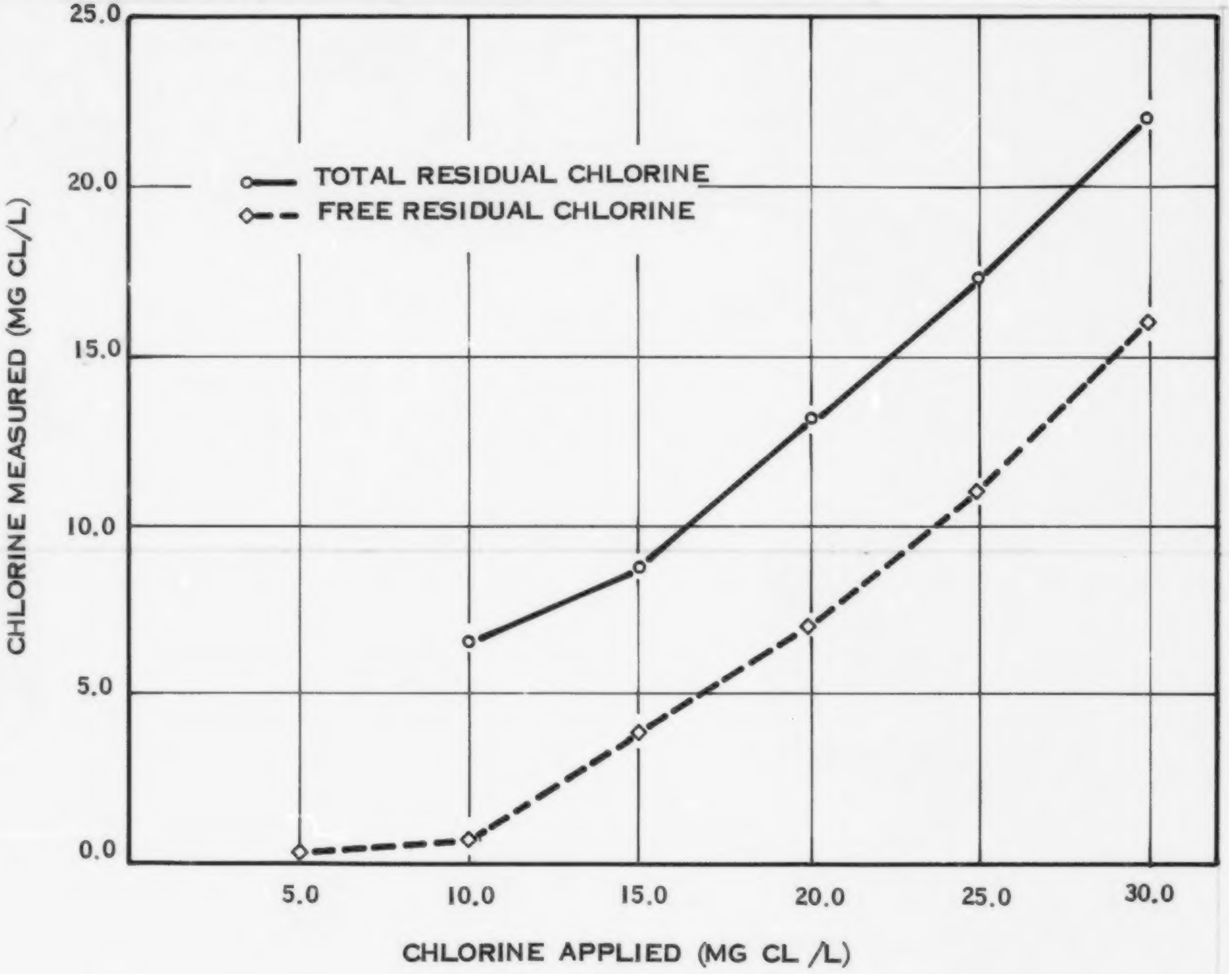


FIGURE B2.

Total and Free Residual Chlorine Concentration measured as a function of applied chlorine after a thirty minute holding time. Media diluted 1/100. T = 25°C. pH = 6.9.

APPENDIX C

DETAILS OF GARDEN GROVE SANITARY SURVEY AND MAINTENANCE PROGRAM

City of Garden Grove

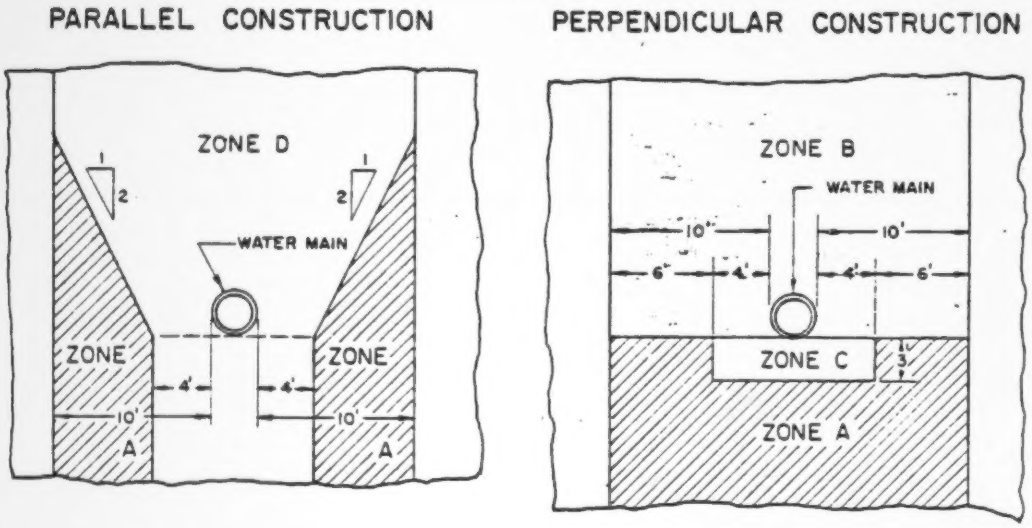
3-17 Chlorination of Completed Pipeline.

All new pipelines and appurtenances shall be flushed and disinfected in accordance with the provisions of AWWA C601 (latest issue). Chlorination shall be accomplished by using a mixture of chlorine gas and water or by chlorine gas fed directly through proper devices. The minimum residual chlorine content after 24 hours shall be 25 parts per million.

Taps and devices for testing, chlorination and flushing shall be placed at locations shown on the plans or as directed by the Director. The abandonment of these devices shall be inspected by the Director prior to backfilling.

After lines have been flushed, samples shall be taken of the water in the lines by a State Certified Laboratory for bacterial analysis in accordance with AWWA specifications. In the event that the bacterial analysis indicates an unsatisfactory condition, chlorination shall be repeated until a satisfactory condition of the water within the pipe is established.

All costs in connection with testing, chlorination and flushing shall be included in the unit price bid per lineal foot of water main installed.


PARALLEL CONSTRUCTION
PERPENDICULAR CONSTRUCTION
ZONE D
1
2
1
2
WATER MAIN
ZONE A
ZONE A
4'
4'
10'
10'
ZONE B
WATER MAIN
10'
10'
6'
4'
4'
6'
ZONE C
3'
ZONE A

IF A MAIN LINE SEWER CANNOT BE LOCATED TEN OR MORE FEET FROM A PRESSURE WATER MAIN AND MUST BE LOCATED WITHIN ANY OF THE ABOVE INDICATED ZONES, SPECIAL CONSTRUCTION WILL BE REQUIRED AS SHOWN BELOW.

IF A MAIN LINE SEWER MUST CROSS A PRESSURE WATER MAIN WITHIN ANY OF THE ABOVE INDICATED ZONES, OR IF A HOUSE LATERAL MUST CROSS IN ZONE B, SPECIAL CONSTRUCTION WILL BE REQUIRED AS SHOWN BELOW.

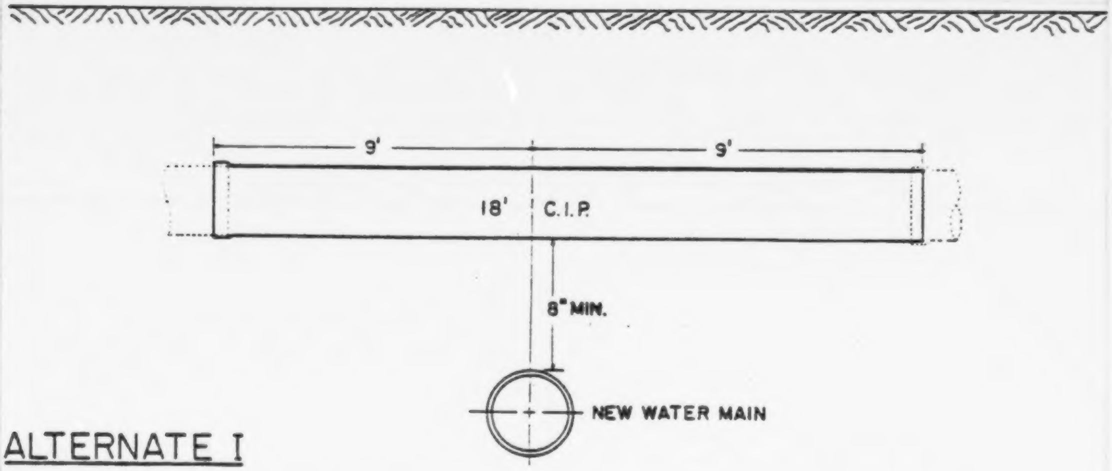
ZONE	SEWER CONSTRUCTION REQUIRMENTS
A	V.C.P. WITH MECHANICAL COMPRESSION JOINTS.
B or C	C.I.P. WITH APPROVED MECHANICAL JOINTS, OR V.C.P. WITH SPECIAL CONCRETE ENCASEMENT PER B-760 ; OR V.C.P. GROUTED IN CONTINUOUS STEEL CASING.
D	DO NOT LOCATE ANY PARALLEL SEWER IN THIS AREA WITHOUT PUBLIC WORKS & DEVELOPMENT DEPARTMENT APPROVAL

FORCE MAINS

PARALLEL CONSTRUCTION NOT PERMITTED IN ANY ZONE
PERPENDICULAR CONSTRUCTION IN ANY ZONE REQUIRES C.I.P. WITH APPROVED MECHANICAL JOINTS; OR ASBESTOS CEMENT PIPE WITH SPECIAL CONCRETE ENCASEMENT

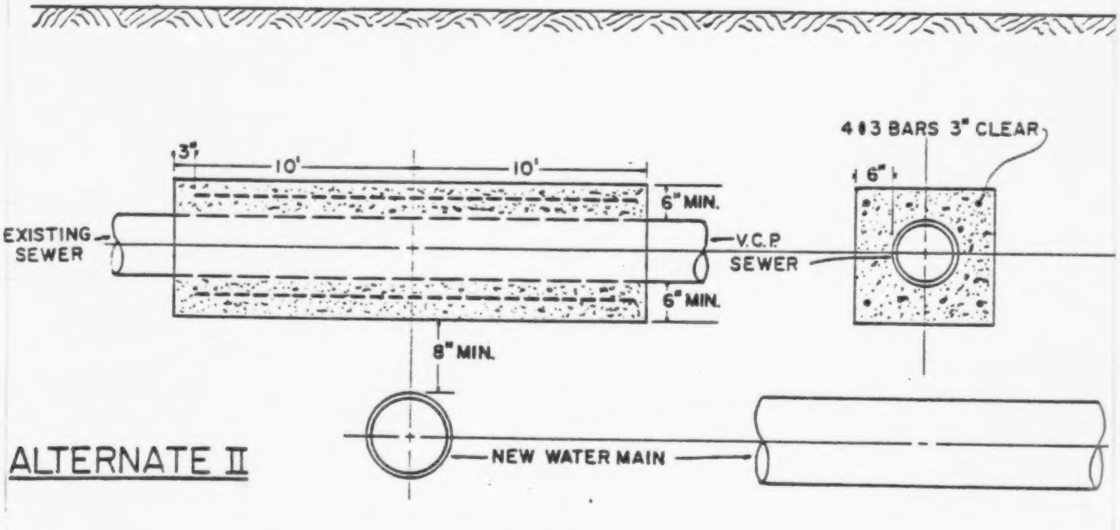
FIGURE C1.

Sewer main details near water mains.
Std. Plan B-762


9'
9'
18'
C.I.P.
8" MIN.
NEW WATER MAIN

ALTERNATE I

REMOVE EQUAL DISTANCE OF EXISTING V.C.P. SEWER LINE ON BOTH SIDES OF CENTER LINE OF WATER MAIN TO ALLOW THE INSTALLATION OF 18 FEET OF BELL & SPIGOT CAST IRON PIPE IN PLACE OF THE EXISTING V.C.P. SEWER LINE. NO CONCRETE ENCASEMENT NECESSARY.


3"
10'
10'
6" MIN.
EXISTING SEWER
V.C.P SEWER
6" MIN.
4 #3 BARS 3" CLEAR
6"
8" MIN.
NEW WATER MAIN

ALTERNATE II

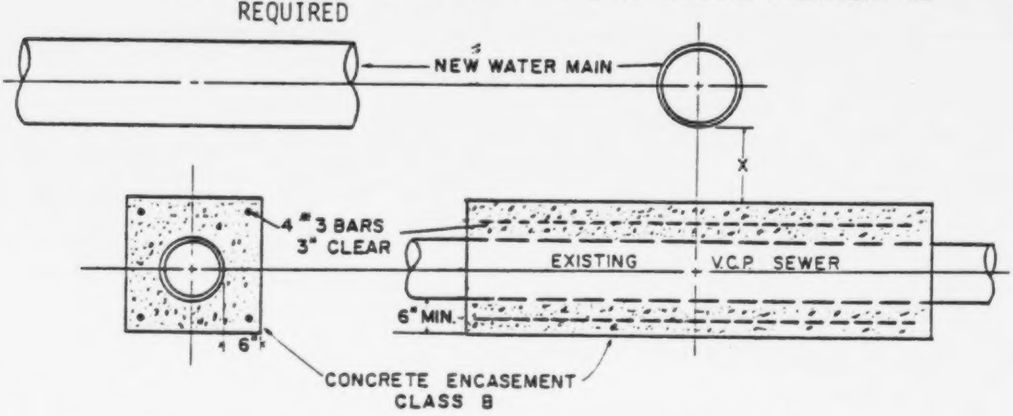
NO SCALE

FIGURE C2.

Water main crossing under sewer lateral.
Std. Plan B-760

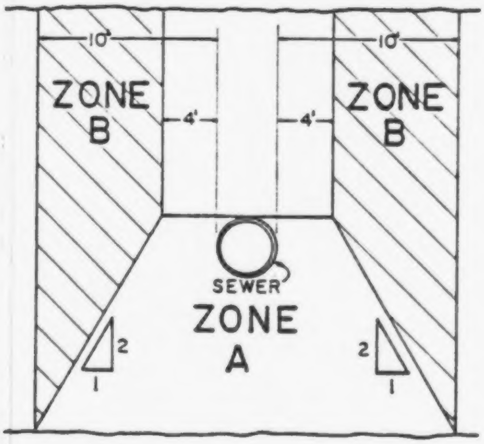
(1) WHEN X IS LESS THAN 3', PROVIDE CONCRETE ENCASEMENT FOR ENTIRE SEWER MAIN WITHIN 10' OF THE WATER MAIN.

(2) WHEN X IS GREATER THAN 3', NO SPECIAL TREATMENT IS REQUIRED


NEW WATER MAIN
X
4 #3 BARS
3" CLEAR
EXISTING V.C.P. SEWER
6" MIN.
6"
CONCRETE ENCASEMENT
CLASS B

NOTE: WHEN A WATER MAIN CROSSES UNDER AN EXISTING MAIN LINE SEWER, CONSTRUCT PER STD. DWG. B-760

PERPENDICULAR CONSTRUCTION


10'
10'
ZONE B
4'
4'
ZONE B
SEWER
ZONE A
2
1
2
1

DO NOT CONSTRUCT ANY PARALLEL WATER MAIN IN ZONE A.

NO WATER MAIN SHALL BE CONSTRUCTED IN ZONE B WITHOUT WRITTEN APPROVAL FROM THE PUBLIC WORKS AND DEVELOPMENT DEPARTMENT.

PARALLEL CONSTRUCTION

FIGURE C3.

Water main details near sewer mains.
Std. Plan B-761